The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

Biochemical Studies on Carbohydrates

CLXI. Paper Partition Chromatography of Sugars, in particular of Hexosamines and Hexuronic Acids

Second Report

By

Hajime Masamune and **Zensaku Yosizawa**

(正宗 一) (吉澤 善作)

From the Medico-chemical Institute, Tohoku University, Sendai

(Received for publication, May 1, 1953)

Paper partition chromatography of sugars was attempted this time in the descending way, which served the purpose more than the ascending (Cf. Masamune and Maki[1]). Of several solvents examined, *n*-butanol-pyridine-water (5:3:2) of Yosizawa[2] and *n*-butylacetate-acetic acid-ethanol-water (3:2:1:1) were found most suitable for irrigation. Since the solvents were let drop from the lower edge of a paper strip, R_r (flow rate of a sugar as compared with rhamnose) values were measured instead of R_f (flow rate of a sugar as compared with solvent front) values. Quite constant R_r values were given within the temperature limits of 22–27°C.

Experimental

Apparatus and Technique

a) In a cylindrical glass jar, 65 cm. long and 18 cm. diametral and having a ground stopper, is placed one of the glass stands which are diagrammatized in Fig. 1. The stands are formed of a 55 cm. rod with a horizontal trident as a foot and a cage with a hexagonal upper margin of 5 cm. sides (Stand I) or a plate (Stand II) attached to the upper end of the rod to support a half Petri dish (diameter 10 cm., hight 2 cm.) or a rectangular glass dish (width 7 cm., length 13 cm., hight 5 cm.) for the solvent.

b) *Chromatograms.* Toyo filter paper No. 2 is cut into strips of 60 cm. × 5 cm. for Stand I, but into strips of 60 cm. × 5–10 cm. for the other stand.

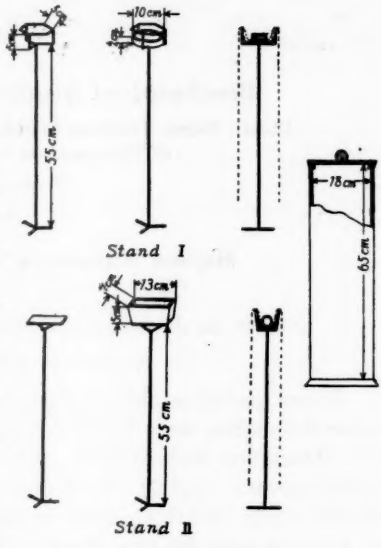
c) *Sampling.* Of a solution, containing a sugar or sugars in 2% or a like concentration with respect to each individual sugar and acidulated to pH 5.8 with a dilute (0.1 *N*) H_2SO_4 (HCl is used for hexosamine hydrochloride solutions), is pipetted 0.015 cc. on a paper strip at a distance

of 7–10 cm. from the upper edge. Each spot covers an area 0.8 cm. in diameter.

d) 30–50 cc. of a solvent are placed in the trough at first and supplemented from time to time during irrigation.

e) *Solvents for irrigation.* Amyl-alcohol-pyridine-water (7:7:6) of Werner and Odin[3] (I), *n*-butanol-pyridine-water (5:3:2) of Yosizawa[2] (II), *n*-butylacetate-acetic acid-water (3:2:1) of Yosizawa[2] (III); III-methanol (6:1) (IV); III-ethanol (6:1) (V); III-*n*-butanol-methanol (6:1:1) (VI); III-*n*-butanol-methanol (6:2:1) of Masamune and Maki[1] (VII).

Fig. 1



f) *Indicators.* Aniline hydrogen phthalate reagent of Partridge[4]; 0.2% ninhydrin in butanol. The latter was applied to hexosamines.

g) *Carbohydrates tested.* 1) L-Arabinose, Takeda.

2) Xylose, Takeda.

3) L-Fucose (crystalline), kindly supplied by Prof. N. Hiyama of Hirosaki University.

4) Rhamnose, Takeda.

5) Galactose, E. Merck.

6) Glucose, E. Merck.

7) Mannose, Takeda.

8) Glucosamine hydrochloride (crystalline)

9) Chondrosamine hydrochloride (crystalline)

10) N-Acetylglucosamine, prepared according to Zuckerkandl and Messiner-Klebermass.[5]

11) N-Acetylchondrosamine prepared according to Masamune, Maki and Hiyama.[6]

12) Glucosamine sulfate. i) The crystalline. 1 g. of chitin was refluxed with 30 cc. of 6 *N* H_2SO_4 for 50 hours. The hydrolysate was freed from most of H_2SO_4 with baryta (The solution was retained on the acid side) and distilled in vacuo to about 10 cc., and 2 volumes of alcohol added. The centrifuged supernatant was condensed furtheron. Half-way tetragonal and hexagonal plates began to appear, when the distil-

lation was stopped and a little additional alcohol was added. The mixture was stood in an ice-chest overnight, and the deposit collected on a funnel with suction and washed with chilled alcohol. The crude product (0.35 g.) was recrystallized once from a dilute H_2SO_4 solution (1%) and three times from water by the aid of alcohol. It browned at about 175°C and decomposed (no liquefaction) at 200°C. Elson-Morgan and Fehling positive. Sulfate ions detected (as $BaSO_4$). N (micro Kjeldahl) 6.2% (Calc. for $C_6H_{13}O_5N \cdot {}^1/_2H_2SO_4$, 6.14%); $[\alpha]_D^{14} = \frac{1.87 \times 100}{2.02 \times 1} = +92.6°$ (at the 3rd minute after dissolution) $\longrightarrow \frac{1.37 \times 100}{2.02 \times 1} = +67.8°$ (at equilibrium).

ii) A condensate. A chitin hydrolysate as the one above was freed from most of H_2SO_4 (Baryta was added to pH 5.8) and distilled in vacuo to a small volume.

13) Chondrosamine sulfate. i) The crystalline. Chondroitinsulfate of Jorpes was electrodialyzed and 2 g. of the free acid recovered were hydrolyzed as above. Then, after liberation from most of H_2SO_4, the hydrolysate was distilled in vacuo to about 10 cc., and 3 volumes of alcohol added. The deposit here was discarded and the mother fluid was condensed to a syrup, taken up in a mixture of methanol and ethanol and condensed over again to a small volume, whereby crystals in petal form began to appear. After dilution with some methanol, the mixture was stood in an ice chest. The crystalline deposit was separated and washed with chilled methanol. Yield 0.15 g. Elson-Morgan and Fehling positive. Neuberg-Saneyoshi negative. Sulfate ions detected. It turned brown at about 175°C and decomposed at 185–186°C with a little gas evolution. N (micro Kjeldahl) 6.3% (Calc. for $C_6H_{13}O_5N \cdot {}^1/_2H_2SO_4$, 6.14%). $[\alpha]_D^{14} = \frac{1.40 \times 100}{1.67 \times 1} = +83.8°$ (at the 5th minute after dissolution) $\longrightarrow \frac{1.10 \times 100}{1.67 \times 1} = +65.9°$ (at equilibrium). ii) A condensate. The chondroitinsulfate was hydrolyzed and most H_2SO_4 was precipitated with baryta followed by concentration of the filtrate as in 11)ii). iii) The amorphous. An amorphous product was prepared according to Masamune and Maki[1] from crystalline chondrosamine hydrochloride.

To prepare chondrosamine sulfate from the hydrochloride, one must be cautious not to use an anion exchange resin to eliminate HCl, because the resin decomposes (probably polymerizes) the hexosamine and the product neutralized with sulfuric acid gives a chromatographic spot with a R_r value even lower than the less mobile spot of chondrosamine sulfate (Cf. Table I). The same was the case with glucosamine.

14) Glucuronic acid (crystalline) was prepared from menthol-glucuronic acid according to Masamune.[7]

15) Barium galacturonate. A crude preparation of barium galacturonate from beet pectin was precipitated from water with 5 volumes of alcohol. The deposit once dried was again taken up in water, decolorized with active charcoal and after passing through a cation exchange resin "Amberite IR-120," neutralized with baryta, followed by reprecipitation and washing with alcohol. The product, dried to constant in a vacuum desiccator (H_2SO_4), analyzed:

Ba (micro Pregl)
8.085 mg. substance, 3.627 mg. $BaSO_4$.
$C_6H_9O_7 \cdot {}^1/_2Ba$. Found, 26.4%; calc., 26.2%.

The substance was freed from barium by means of the cation exchange resin before use.

16) Barium mannuronate, prepared by combining Nelson-Cretcher[8] and Link-Nedden[9] method.

Ba (micro Pregl)
5.251 mg. substance, 2.371 mg. $BaSO_4$.
$C_6H_9O_7 \cdot {}^1/_2Ba$. Found, 26.6%; calc., 26.2%.

This was also freed from barium similar to above for the chromatography.

17) Lactose, E. Merck.

18) Mucosin sulfate, prepared as described by Masamune and Maki.[1]

Results

Table I and Fig. 2 show the mean R_r value of sugars run by the various solvents at 22–27°C for 24 hours. Within so wide a range of temperature the R_r value of a sugar by one and the same solvent did not vary more than 0.03.

Following findings were particularly to be pointed out:—

On the chromatograms of hexosamine salts. a) Hexosamines were difficultly separated by the solvents when they were applied as hydrochlorides (Cf. Table I).

b) When crystalline glucosamine sulfate (12)i)) was run, a spot of higher R_r was obtained by most of the solvents, while the condensate (12) ii)) gave a spot of lower R_r in addition. Either of them gave rise to a spot corresponding to free glucosamine barely (These faint spots are not shown in Figs. 3 and 4).

Remark. When the solutions were neutralized or slightly alkalized, the spots of lower R_r became weaker and those of higher R_r as well as those corresponding to free glucosamine stronger.

c) Crystalline chondrosamine sulfate (13)i)) gave always only one spot of lower R_r. But the condensate (13)ii)) gave two spots, the

TABLE I
Average R_r Values of Sugars

TABLE I

Average R_r Values of Sugars run by Various Solvents

Temperature of test, 22–27°C; duration of test 24 hrs. Indicator, aniline-hydrogen phthalate of Partridge

No.	Solvent Composition	Mean movements of rhamnose (spot center) from the start* cm.	Average R_f values of sugars																	
			L-Fucose	Xylose	L-Arabinose	Mannose	Glucose	Galactose	Fructose	Glucosamine hydrochloride†	Chondrosamine hydrochloride†	Glucosamine sulfate‡	Chondrosamine sulfate‡	N-Acetylglucosamine	N-Acetylchondrosamine	Glucuronic acid§	Galacturonic acid	Mannuronic acid§	Mucosin sulfate	Lactose
I	Amylalcohol 7 Pyridine 7 Water 6	39	0.87	0.86	0.76	0.76	0.67	0.60	0.75	0.58	0.54	0.51 0.18	0.47 0.13	0.85		0.26 (0.86)	0.22	0.30	0.21	0.49
II	*n*-Butanol 5 Pyridine 3 Water 2	43	0.82	0.81	0.70	0.68	0.59	0.53	0.69	0.43	0.38	0.42 0.05	0.34 0.02	0.81		0.18 (0.90)	0.12	0.21	0.06	0.28
III	*n*-Butylacetate 3 Acetic acid 2 Water 1	40	0.85	0.72	0.68	0.57	0.49	0.46	0.65	0.27	0.26	0.26 0.11	0.16 0.06	0.83	0.84	0.41 (0.72)	0.38	0.47 (0.78)	0.19	0.21
IV	*n*-Butylacetate 3 Acetic acid 2 Methanol 1 Water 1	45	0.83	0.72	0.67	0.55	0.48	0.44	0.62	0.28	0.26	0.27 0.11	0.23 0.07	0.83	0.81	0.39 (0.70)	0.35	0.48 (0.80)	0.15	0.17
V	*n*-Butylacetate 3 Acetic acid 2 Ethanol 1 Water 1	46	0.86	0.69	0.65	0.55	0.45	0.42	0.59	0.19	0.20	0.23 0.07	0.14 0.04	0.78	0.77	0.38 (0.68)	0.32	0.46 (0.79)	0.15	0.15
VI	*n*-Butylacetate 3 Acetic acid 2 Butanol 1 Methanol 1 Water 1	43	0.82	0.71	0.63	0.52	0.44	0.39	0.60	0.24	0.22	0.25 0.08	0.22 0.06	0.79		0.33 (0.64)	0.27	0.47 (0.79)	0.12	0.14
VII	*n*-Butylacetate 3 Acetic acid 2 Butanol 2 Methanol 1 Water 1	34	0.76	0.61	0.55	0.43	0.34	0.29	0.47	0.16	0.14	0.16 0.04	0.14 0.02	0.65	0.61	0.24 (0.57)	0.18	0.42 (0.76)	0.04	0.07

* The variation ±5 cm. † The spot tailed often by Solent I. By most of the solvents one spot only was given distinctly with a very weak spot corresponding to free glucosamine besides. ‡ Two spots appeared, though not always depending on the preparation examined, and they tailed often by Solvent I. § The figures in parentheses express R_r values of the lactone.

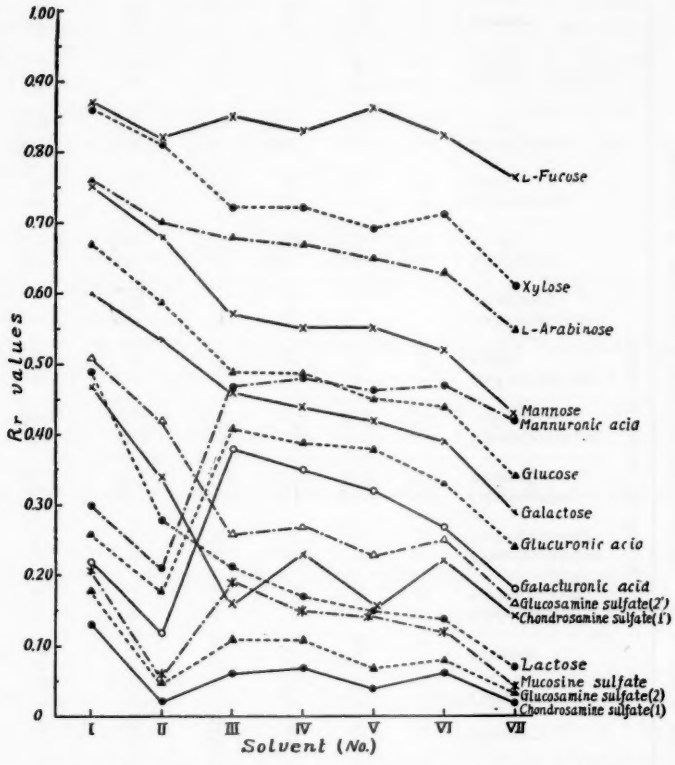


Fig. 2. A chart showing the average R_f values of sugars in Table I. (1') and (2') : the quicker moving spots respectively of chondrosamine sulfate and glucosamine sulfate, whose slower moving spots are (1) and (2).

spot of higher R_f dominating in the cases of Solvents I and II and the one of lower R_f in the cases of Solvents III–VII. The preparation in 13) iii) resembled that in 13) i) in this connection, although the spot of higher R_f was not missing in chromatograms.

Remark. When the chondrosamine sulfate solutions which were of pH 5.8, were neutralized or slightly alkalized, a spot of higher R_f appeared or enlarged and, besides, a distinct spot of free chondrosamine occurred particularly in case of the preparations in 13) ii) and 13) iii).

On the chromatograms of hexuronic acids. As is demonstrated in Table I, Solvent II is most appropriate for separation of the three hexuronic acids, but Solvent V is also expedient if mannuronic acid which has not as yet been discovered in animal materials, is not present in the unknown

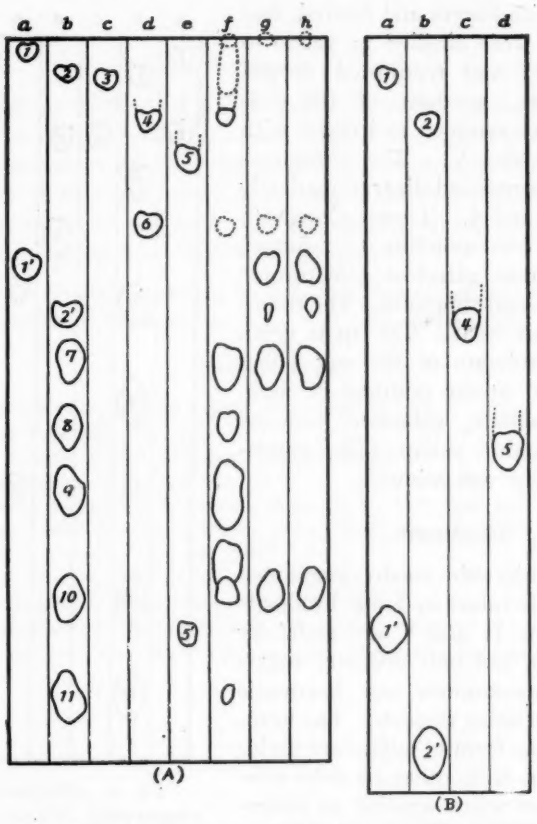


Fig. 3. *n*-Butanol-pyridine-water (5:3:2) chromatograms developed with aniline hydrogen phthalate. Temperature of test: 22–27°C. Duration of test: (A) 24 hrs.; (Ba) and (Bb) 3×24 hrs.; (Bc) and (Bd) 4×24 hrs.
a, b, c, d and e: standard runs. f: traganth (a 1 *N* H_2SO_4-hydrolysate). g and h: 1 *N* H_2SO_4 hydrolysates of mucinogens of toad and frog egg-covering respectively. 1 and 1′: chondrosamine sulfate (13)ii)), 2 and 2′: glucosamine sulfate (12)ii)), 3: mucosin sulfate, 4: galacturonic acid, 5: glucuronic acid, 5′: glucuronolactone, 6: lactose, 7: galactose, 8: glucose, 9: mannose, 10: L-fucose, 11: rhamnose.

substance (Cf. also Figs. 2–4).

Traganth[10] *and mucinogen of toad and frog egg covering.*[11] These substances were examined, as examples, by the device in this account. 50 mg. of the substances were heated with 5 cc. of 1 *N* H_2SO_4, as sealed in an ampoule, at 100°C for 5 hours. The hydrolysates were neutralized

to pH 5.8 with baryta and filtered, and the filtrates were distilled in vacuo to about 0.5 cc. and readjusted to pH 5.8. 0.02 cc. portions of the condensates were sampled to irrigate with Solvents II and V. The chromatograms are reproduced diagrammatically in Figs. 3A and 4. Those of traganth show spots corresponding to L-fucose, xylose, arabinose, galactose, galacturonic acid, glucose and rhamnose. The rhamnose spot was weak. The spots given by the hydrolysate of the egg jellies were located at the positions of chondrosamine sulfate, galactose, L-fucose and glucosamine sulfate (The glucosamine content was minute).

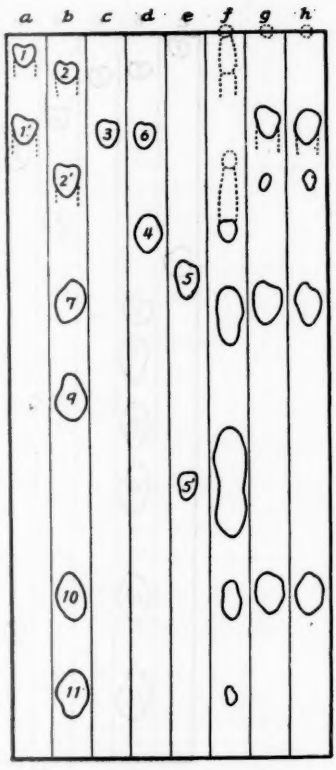


Fig. 4. *n*-Butylacetate-acetic acid-ethanol-water (3:2:1:1) chromatograms developed with aniline hydrogen phthalate. Temperature of test, 22–27°C. Duration of test 24 hrs.

Characters and ciphers signify the same as in Fig. 3.

Comments

The noticeable results mentioned as well as R_f values in Table I indicate that Solvents II and V are useful for separation of not only ordinary sugars but also hexosamines and hexuronic acids in descending manner. The hexosamines in the form of sulfate are divided well from each other by those solvents, but not when applied as hydrochlorides. Solvent II which is alkaline causes partial decomposition of hexosamines as is already known, nevertheless it is advantageous for elear-cut separation of their sulfates by long-timed irrigation (Fig. 3B). Solvents IV–VII are prepared by addition of an alcohol or alcohols to Solvent III. Of the alcohols, methanol was found to spread and obscure spots. Ethanol and butanol contrariwise locarized sugars into distinct spots, while the latter retarded the flow of sugars entailing difficult chromatography. Thus Solvent V containing ethanol is most efficient.

By the two solvents II and V, the disaccharide unit, remaining uncleft after hydrolysis of an itin or itinsulfuric acid with 1 *N* H_2SO_4, is separated far from the hexuronic acid and hexosamine, especially when the test

is prolonged.

Summary

A descending method of paper partition chromatography of sugars has been devised,where solutions of sulfate of hexosamines and non-amino sugars are adjusted to pH 5.8 with H_2SO_4 and irrigated with *n*-butanol-pyridine-water (5:3:2) or *n*-butylacetate-acetic acid-ethanol-water(3:2:1:1).

Chromatograms of traganth and mucinogen of toad and frog egg-covering (hydrolysates) were given as examples.

Through the Grant Committee for Scientific Researches was given a grant from the Education Department in aid to us. H. Masamune.

References

1) Masamune & Maki, Tohoku J. Exp. Med., 1952, **55**, 299.
2) Yosizawa, *ibid.*, 1950, **53**, 125.
3) Werner & Odin, Upsala Läkareför. Förh., 1949, **54**, 69.
4) Partridge, Nature, 1949, **164**, 443.
5) Zuckerkandl & Messiner-Klebermass, Bioch. Zeits., 1931, **236**, 21.
6) Masamune, Maki & Hiyama, Tohoku J. Exp. Med., 1951, **54**, 313.
7) Masamune, J. Bioch., 1933, **18**, 259.
8) Nelson & Cretcher, J. Am. Chem. Soc., 1929, **51**, 1918.
9) Link & Nedden, J. Biol. Chem., 1931–32, **94**, 307.
10) Cf. Maki, Thoku J. Exp. Med., 1952, **55**, 324 (Fig. 4).
11) Cf. Hiyama, *ibid.*, 1949, **50**, 373, 379 and 385; Masamune, Yosizawa & Maki, *ibid.*, 1951, **53**, 237.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

A Simple Method of Visualizing the Acid Secretion from the Gastric Mucosa*

By

Kiyotaka Takahashi, Tsuyoshi Aoki and **Masao Wada**
(高橋 清孝) (青木 健) (和田 正男)

From the Physiological Laboratory of Prof. M. Wada,
Tohoku University School of Medicine, Sendai

(Received for publication, December 10, 1953)

In order to investigate precisely the mechanism of gastric acid secretion and to analyze the actions of antacid agents, we have devised a simple method for visualizing minute amounts of the acid secretion from the gastric mucosa. The area of the mucosa to be studied is painted with Carbowax 1500W in which Congo red has been dissolved in 5 to 10 per cent. This reagent is suitable for showing the presence of the HCl of pH 1.0 to 2.3. When the acid secretion occurs, a bluish black spot appears at each active foveola of the mucosa through a red transparent layer of the reagent (Fig. 1). The appearance of the spots can be detected under the microscope or with a magnifying lens; the secretion of the order of 0.00001 ml may easily be seen as a spot with the naked eye. The greater the amount of the secretion the larger is the size of the spots. If the secretion is abundant, the coalescence of the spots may be produced. The simplicity and usefulness of this method have been demonstrated in the experiments on the gastric mucous membrane, *in situ* or exteriorized, of frogs, toads, cats and dogs.

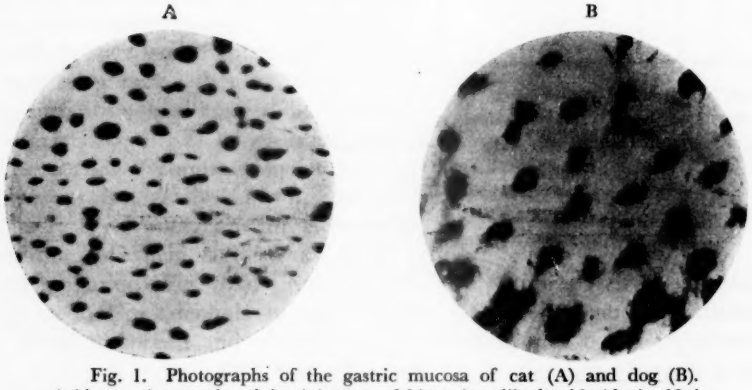


Fig. 1. Photographs of the gastric mucosa of cat (A) and dog (B). Acid secretion produced by injection of histamine dihydrochloride in 10^{-4} under the mucosa was visualized as black spots at active foveolae. Magnification: ×50.

* The technique was demonstrated at the 30th Annual Meeting of the Japanese Physiological Society, Fukuoka, April 2, 1953.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1954

Velocities of Spreading Induction in Human and and Mammalian Retinas

By

Koiti Motokawa, Kituya Iwama and **Mituru Ebe**
(本川 弘一) (岩間 吉也) (江部 充)

From the Physiological Laboratory of Prof. K. Motokawa, Tohoku University, Sendai

(Received for publication May 1, 1953)

Introduction

In previous papers by Motokawa,[4,5] it was shown that a physiological state termed "indirect induction" can be detected by the method of electrostimulation around a retinal area illuminated by colored light, and that such a physiological state remains almost unchanged for a period of about 20 seconds after removal of inducing light. In the preceding paper by Motokawa[6] it was further suggested that such a stationary change in the retina would be established by a certain process propagated from the illuminated retinal area into the surrounding areas.

The present investigation was directed primarily towards measuring the velocity of this spreading process.

Experimental

Method

Measurements were made on decerebrate cats and on man. Animals were decerebrated under ether narcosis. A small dose of urethane, from 2 to 4 cc. of 25 per cent solution, was injected intraperitoneally to suppress reflex movements during experiments. After the cornea and lens of the eye had been removed, a microelectrode of Granit's type was placed onto the retina to pick up spike responses to light or an electric stimulus. Responses were detected with a cathode ray oscillograph and a loud-speaker. As it was necessary to pick up discharges from a large group of retinal elements, the electrode used was of relatively large size, that is, 60 to 100 μ in diameter.

For electrical stimulation of the retina, a pair of non-polarizable electrodes of Zn-$ZnSO_4$-agar type was used. While the reference electrode was held in place at the injured portion of the brain, the other electrode 1 mm. in diameter, together with the pick-up electrode, was inserted into the eyebulb by means of a micromanipulator in such a manner that the

tip of the stimulating electrode came to a distance of about 5 mm. from the retinal surface when the pick-up electrode just reached the retina. The stimulating electrode was usually made cathodal, but with stimulating currents of reverse direction, similar results could be obtained.

The duration of the electric stimulus was 0.5 seconds. The threshold voltage was between 0.5 and 3 volts. As the total resistance of the stimulating circuit was about 30,000 ohms, a current of about 20 to 100 μA was sufficient to evoke spike discharges. Beginning with a voltage high enough to elicit a distinct response, the stimulating voltage was reduced step by step until two observers agreed that no response was perceptible.

Spontaneous discharges were always perceived on the screen of the oscillograph, and their amount was found to be a sensitive indicator showing how close the microelectrode was to the retina. If the electrode happened to be displaced owing to some mechanical disturbance such as movement of the animal, the amount of spontaneous discharge perceptible on the screen decreased markedly, and at the same time a rise in threshold took place. In such cases the electrode was adjusted until the spontaneous noise attained the same level as before. Then the value of threshold also would be restored to the initial level.

Success or failure in our experiments depended solely upon keeping the retina sensitive enough to electrical stimulation while avoiding reflex movements of the animal.[8] Too deep anesthesia tends to suppress electrical excitability, in particular, supernormal excitability after illumination. Some barbiturates were found objectionable because they strongly reduced the electrical excitability of the retina. The use of tubocurarin. for suppression of reflex movements was abandoned for the same reasone Urethane seemed to be preferable to the other anesthetics tested sofar.

It was usually observed that toward the end of an experiment which had lasted for a few hours, the phenomenon of retinal induction disappeared first, followed by that of supernormal excitability after illumination, the loss of electrical excitability in general and the disappearance of responses to photic stimulation in order, as the general condition of the experimental animal became worse. This suggests that animals in good condition are needed for investigation of retinal induction.

The method for human experiments is described in previous papers by Motokawa.[3,7] Optical arrangements and detailed procedures will be mentioned together with the results. All measurements were performed after a preliminary dark adaptation of about 20 minutes.

Results

1. Retinal induction and its neutralization in cat's retina

Records in Fig. 1 illustrate supernormal electrical excitability after

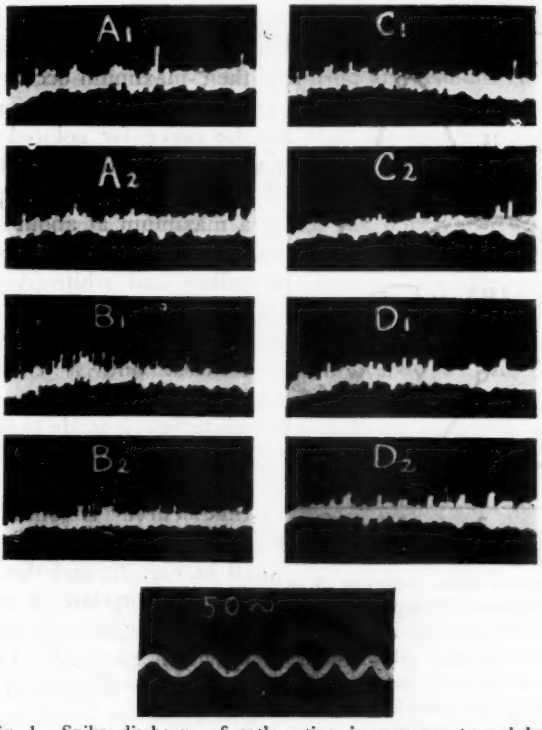


Fig. 1. Spike discharge of cat's retina in response to subthreshold electrical stimulation after a conditioning stimulus, white light of 2 seconds. A_1, B_1, C_1 and D_1 represent responses to electric stimuli 20% below threshold applied 1, 2, 3 and 4 seconds after termination of white light. A_2, B_2, C_2 and D_2 are respective controls. In controls, stimuli 80% below threshold were used.

a conditioning white light stimulus. A_1, B_1, C_1 and D_1 represent responses to an electric stimulus 20 per cent below threshold 1, 2, 3 and 4 seconds respectively after cessation of 2 second's illumination, while A_2, B_2, C_2 and D_2 are respective controls. The record B_1 shows a well perceptible response indicating that a supernormality of about 20% prevails 2 seconds after the illumination. The other records show no remarkable responses as compared with controls so that supernormality may be below 20%.

If ζ-values or percentage increases in electrical sensitivity are plotted against time after removal of pre-illuminating light, a ζ-time curve having a maximum at about 2 seconds will be obtained for white light (broken curves in Fig. 2). When red, yellow, green and blue lights are used for pre-illumination, the crest time of the curve lies at about 1, 1.5, 2 and 3

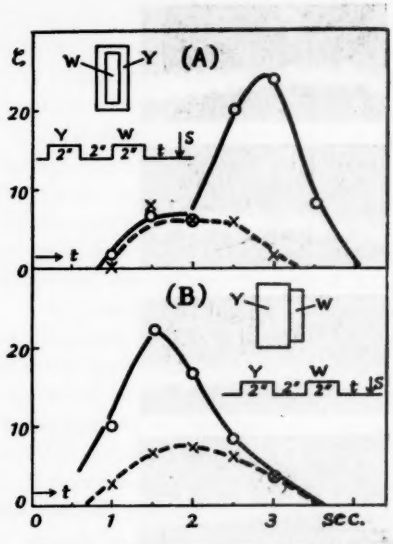


Fig. 2. Direct or successive induction (A) and indirect or simultaneous induction (B) in cat's retina. Ordinates: ζ or percentage increases in electrical sensitivity above resting level following illumination. Abscissas: time after termination of white light. Broken curves represent ζ-time curves for white light alone, and continuous ones those for successive stimuli, yellow and white. Arrangements of patches and temporal sequence of procedure are shown in insets, where Y, W, S and t denote yellow light, white light, an electric stimulus of 0.5 sec. and the interval between W and S respectively. The intensity of white light was 300 lux.

seconds respectively.[8] If the white light is preceded by colored light, the ζ-maximum occurs at the time corresponding to the maximum of the curve for colored light complementary to the inducing light used. For example, a curve having a maximum at about 3 seconds is obtained for successive stimuli, yellow and white (A in Fig. 2). This phenomenon is a physiological correlate of successive color contrast and is called "direct induction." If the subsequent white light is made to fall on a part adjacent to the retinal area pre-illuminated by yellow light, a ζ-time curve is obtained whose maximum lies at about 1.5 seconds (B in Fig. 2), and this is a physiological correlate of simultaneous contrast for reasons stated in the previous paper.[4] This phenomenon is designated "indirect induction" to be set against direct induction.

It was demonstrated in human experiments[6] that the direct induction set up by any colored light is extinguished by exposing the pre-illuminated retinal area to another light complementary to the preceding light. Similarly, the indirect induction established around a retinal image is neutralized by light of the same sort as the inducing one. The indirect induction caused by any colored light at any retinal area disappears when a remote region of the retina is illuminated by another light complementary to the former. A group of such phenomena was designated "neutralization of retinal induction."

In the present investigation this phenomenon was reproduced in animal experiments, because it provides a useful means for measuring the propagation velocity of the spreading process or induction. The patch used in the following experiments consisted of ground glass covered

with a sheet of black paper, from which a figure of varying size and shape was cut. The patch was illuminated from behind with spectral light, colored light from a filter or white light from an incandescent lamp. The light from the patch was reflected by means of a concave mirror in front of the animal's face in such a manner that the image of the patch was focused on the retina. The image was about 1/4 times as large as the patch.

In the experiment shown in Fig. 3, the effect of spectral lights of different wave-lengths upon the direct induction caused by red light from a filter, whose range of transmission was from 610 to 700 mμ, was investigated. The eye was exposed for 2 seconds to red light (R), spectral light (λ) and white light (W) in succession with an interval of 2 seconds between them, and an electric stimulus (S) was delivered just 2.5 seconds after termination of white light. The time of 2.5 seconds corresponds to the crest time of the ζ-time curve which would be obtained for successive stimuli, red and white.

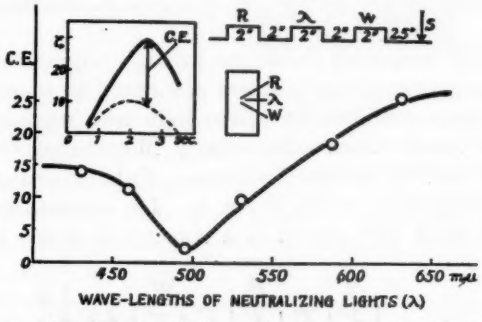


Fig. 3. Neutralization of direct induction set up in cat's retina by red light with spectral lights of various wave-lengths. Ordinates: contrast effects (C. E.), a quantity indicated by a vertical line in inset. Inducing red light (R), neutralizing spectral light (λ) and white test-light (W) were applied to the same region of retina.

As a measure of direct induction served a contrast effect, i.e. an increase of ζ-value at 2.5 seconds above the control or the ζ-time curve for white light alone (cf. inset of Fig. 3). As can be seen in this figure, contrast effects are minimal at the blue-green part of the spectrum which corresponds to the complementary color to the inducing red light. This finding indicates that the spectral light complementary to inducing light is most effective for neutralization.

In the second series of experiments, neutralizing spectral light was presented in juxtaposition so as to neutralize the direct induction pre-

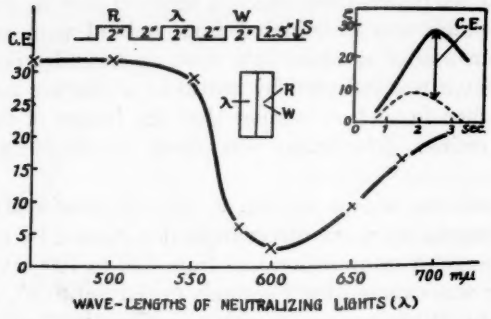


Fig. 4. Neutralization of direct induction set up in cat's retina by red light with indirect induction caused by spectral light. Neutralizing spectral light was presented in juxtaposition.

established by red light with the indirect induction set up by neutralizing light. The data are shown in Fig. 4. In contrast with the data shown in Fig. 3, the orange to red part of the spectrum is effective for neutralization.

2. Velocity of spreading induction in cat's retina

In the following experiment, it was attempted to neutralize the indirect induction pre-established by yellow light with spreading induction originating in a remote area subsequently illuminated by blue light. The patches used and the temporal sequence of photic and electric stimulation are shown diagrammatically in Fig. 5. The combination of a yellow (Y) and a white patch (W) served as a detector to detect the arrival of

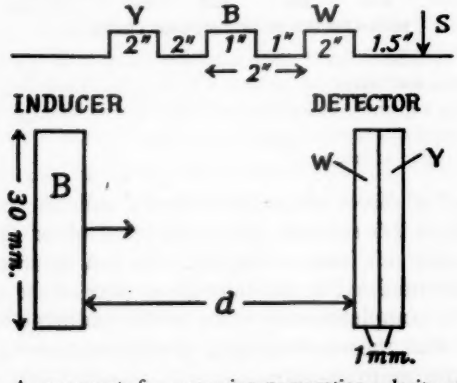


Fig. 5. Arrangements for measuring propagation velocity of spreading induction. B, Y and W are blue, yellow and white patches presented in a temporal sequence indicated respectively.

the spreading induction initiated by a "inducer" consisting of a blue patch (B). The images of Y and W were focused in the neighborhood of the pick-up electrode in the retina. The time interval between Y and B may be arbitrary, although it was fixed at 2 seconds for convenience. The interval between the onset of B and that of W is of great importance, because it determines whether or not neutralization takes place for a given distance between B and W. This time interval was, however, fixed at 2 seconds, and the distance between B and W was varied instead.

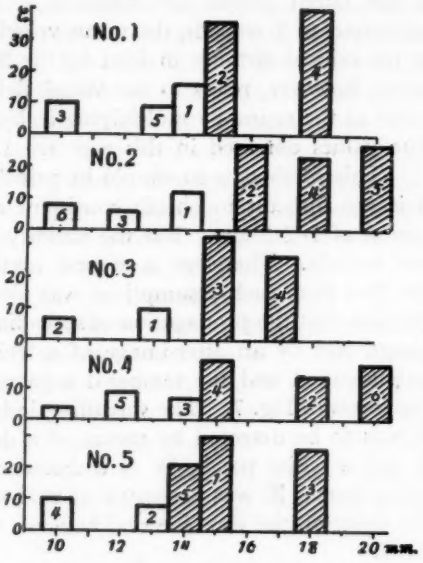


Fig. 6. Data from 5 cats obtained with arrangements shown in Fig. 5. Rectangles represent ζ-values for various distances between B and W. Numbers in rectangles show order of measurements. Higher values of ζ represented by shaded rectangles indicate that spreading induction could not reach the detector in 2 seconds.

In Fig. 6 ζ-values at 1.5 seconds for various distances are expressed by rectangles of varying heights. They are lower than 10 in most cases, so long as the distance is shorter than a certain definite value (see white rectangles in Fig. 6); these values of ζ correspond to the ζ-value for the white test-light alone. From this finding it is very likely that the indirect induction at the detector was neutralized for shorter distances between B and W.

However, ζ-values for distances longer than 15 mm. were as high as 30, corresponding to maximal ζ-values for successive stimuli, Y and W without presentation of B. This indicates that no neutralization took place at the detector. The ineffectiveness of B for longer distances might be considered to mean that the spreading process initiated by B suffered from decrement and could not reach the detector. This explanation is untenable, because neutralization takes place at greater distances, when the time interval between B and W is made longer. It is likely that the arrival of spreading induction at the detector must precede the onset of the white test-light for neutralization to take place. If this supposition is correct, the critical distance for neutralization, e. g. 15 mm. represents presumably a distance traveled by spreading induction in 2 seconds.

If the latent period for initiation of spreading induction is negligible compared to 2 seconds, the mean velocity of spreading induction is equal to the critical distance divided by the fixed time, i. e. 7.5 mm./sec. This value, however, refers to the visual field and must be reduced to the value at the retina by multiplying it by the known reduction factor 1/4. The values obtained in this way are 1.88–2.00 mm./sec.

This method is so simple in principle as well as in procedure, that it is not difficult to obtain consistent results from measurements over a period of 1–2 hours. But the validity of the assumption made above is not certain. Therefore a second method which is more complicated, but free from such assumptions was also used. This method is based on the fact that the propagation of spreading induction is blocked by a retinal image and by an after-image of a white patch.[6] The arrangements of patches used and the temporal sequence of the procedure are shown in the insets of Fig. 7. The spreading induction initiated by a yellow patch, Y was to be detected by means of a detector consisting of a blue patch, B and a white patch, W at a distance of 16 mm. from Y. A blocking white patch, Bl was presented at various points between the inducer and the detector, the time interval between the onset of Y and that of Bl being fixed.

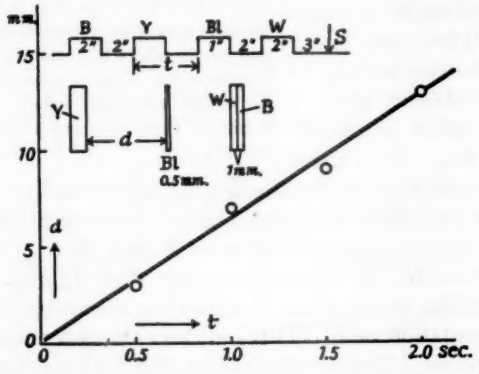


Fig. 7. Relation between time and distance traveled by spreading induction in dark-adapted cat's retina. Y: inducer. B and W: detector. Bl: blocking white patch which was displaced step by step to vary the distance from Y.

When the distance between Y and Bl is too long, spreading induction is blocked at Bl and not able to cause any neutralization at the detector. For too short distances, however, neutralization always occurs, because the moving front of spreading induction has proceeded beyond Bl when

it is presented. A critical distance can thus be determined for a given time interval. Critical distances for time intervals of 0.5, 1.0, 1.5 and 2.0 seconds are plotted against time intervals in Fig. 7. The relation is represented by a straight line passing through the origin, justifying the assumption made above that the latent period of spreading induction sufficiently short relative to time taken for propagation over 15 mm. It is also apparent that the velocity is uniform throughout propagation.

Because these measurements are time consuming we have succeeded in obtaining reliable in only 2 cases. The velocities of spreading induction determined in this way are 1.6 and 1.7 mm./sec. at the retina.

3. Velocity of spreading induction in human retina

Measurements were performed in three normal subjects with the first method. In this experiment a detector consinstig of Y and W was presented at a definite part of the retina. An inducer, B was displaced step by step, the time interval between the onset of B and that of W being fixed at 1.5 seconds. A fixation mark was placed several mm. below the detector. All these patches were presented in a vertical plane 30 cm. in front of the subject. Data obtained are illustrated in Fig. 8. The critical

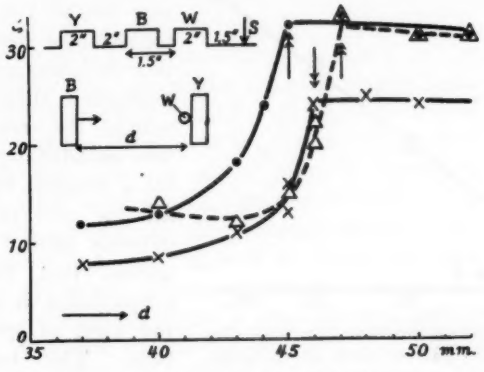


Fig. 8. Measurements of propagation velocity of spreading induction in dark-adapted human retina. B: inducer. Y and W: detector. Double arrows indicate critical distances at which spreading induction initiated by B just reached the detector in 1.5 seconds.

distances indicated by double arrows in this figure are 45, 46 and 47 mm. in three subjects; the critical distances divided by the fixed time of 1.5 sec. are 30.0, 30.6 and 31.2 mm./sec. When the posterior nodal distance of the eye is assumed to be 17 mm., retinal velocities 1.70, 1.72 and 1.77 mm./sec. are obtained. It is to be noted that these values are in close agreement with those obtained from cats.

As can be seen in Fig. 8, the slope of the ζ-distance curve is so steep in the neighborhood of the critical distance that a difference of 1 mm. in distance corresponds to a difference of 8 in terms of ζ. Since the error of measurement of ζ seldom exceeds 2 in trained subjects, the error of measurement must be smaller than 0.5 mm. in terms of distance, corresponding an experimental error of about a few per cent. In cats the error is, however, somewhat greater owing to difficulties in maintaining a steady excitable state of the retina for a sufficiently long period.

In the next series of experiments, more detailed measurements were carried out by the second method in another group of subjects, one of whom was deuteranomalous. The interval between the onset of the inducer and that of the blocking patch was fixed at 0.5, 1.0, 1.5 and 2.0 seconds, and the distance between the inducer and the blocking patch was varied step by step to find a critical distance for each interval fixed.

Three complementary sets of inducer and detector were used in order to investigate the dependence of the velocity upon the kind of spreading induction. The time interval between the white test light and the electrical stimulus was properly chosen, for example, it was made 1.0, 1.5 and 3.0 seconds for red, yellow and blue detectors repsectively. An example is shown in Fig. 9, in which the relation between time and distance is represented by a straight line common to all the sets of inducer and detector used. It is now apparent that the velocity of propagation is independent of the kind of spreading induction. The retinal velocity computed from this relation is 1.7 mm./sec. Exactly the same value was obtained from another normal person. It is to be noted that the value obtained from a deuteranomalous person was found to be equal to the normal value.

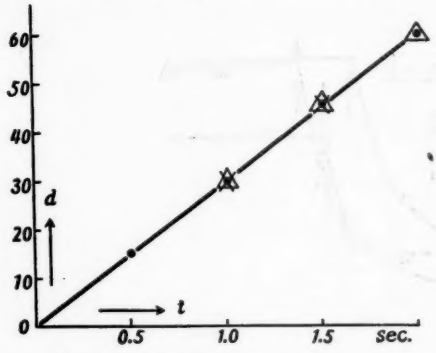


Fig. 9. Relation between time and distance traveled by different kinds of spreading induction in human retina. Dots, crosses and triangles refer to data obtained with yellow, blue and red detectors respectively.

Next, the propagation velocity was measured in different regions of the retina. The point of fixation was displaced step by step to the left by 10°, 20° and 30°, so that the temporal periphery of the retina might be exposed to the patches. In this experiment, contrary to our expectation, the propagation velocity was found to depend little on retinal location.

TABLE I

Retinal Velocities of Spreading Induction in mm. per sec.

Nos. of sub. or animals	1	2	3	4	5	6	7	Average
Man	1.70	1.72	1.77	1.70	1.70	1.70	—	1.72±0.01
Cat	1.88	2.00	1.88	1.88	1.88	1.60	1.70	1.83±0.05

Also no difference could be found between the velocities in the vertical and horizontal directions, although we had expected some difference in view of other facts suggesting anisotropy of the retina with regard to its function.[5]

Discussion

It is obvious that the velocity determined above represents the velocity of the moving front of spreading induction. The moving front leaves a kind of physiological change, our so-called indirect induction behind it. If the propagation of spreading induction is blocked at a certain line in the retina, there can be detected no indirect induction beyond the blocking line.[6] This supports the interpretation that indirect induction is caused by spreading induction.

The intensity of indirect induction measured in terms of contrast effects defined above decreases as the distance from the margin of a retinal image increases.[5] From this fact it is very likely that spreading induction becomes weaker and less effective in causing indirect induction as it propagates. At moderate intensities of the inducer, spreading induction becomes so weak after traversing a distance of about 6° in visual angle, that it can cause no directly detectable induction. Nevertheless this spreading induction can still continue to travel and neutralize another kind of indirect induction pre-established at a greater distance.

In general, indirect induction is unstable and tends to decay spontaneously, and it is due to this unstableness that indirect induction is neutralized by spreading induction of so little energy. On the contrary, a relatively great amount of energy would be necessary for establishing indirect induction anew. This is the reason why spreading induction loses its capacity to establish indirect induction on the way to a distant detector.

In this manner, spreading induction suffers from decrement with regard to intensity or energy. The type of energy transmission must, therefore, be regarded as different from that in nervous conduction which follows the all-or-nothing law.

It is to be noted that in spite of such decrement in energy, the velocity

of spreading induction is uniform throughout propagation. It is also worthy of attention that the velocity depends little on the kind of spreading induction or on the wave-length of the inducer. However, it is only in the dark-adapted retina that we obtain velocities independent of the kind of spreading induction. It has been found that the velocity can be different according to the wave-length of the inducer used, when spreading induction is propagated through a retinal area pre-adapted to colored light. Details about this phenomenon will be reported in a later paper.

Summary

The retinal induction set up by a colored patch around its retinal image disappears when a remote region of the retina is illuminated by another colored light complementary to the former. The mechanism of this phenomenon is such that the retinal induction is neutralized by some spreading process originating in the remote region illuminated. Such a process is called " spreading induction."

The propagation of spreading induction is blocked at an image and at an after-image of a white patch. Taking advantage of this phenomenon, the velocity of spreading induction was measured in 7 cats and in 6 human subjects. The values obtained were on an average 1.83 ± 0.05 mm./sec. in cat's retina and 1.72 ± 0.01 mm./sec. in the human retina. The velocity was found uniform throughout propagation. It depended neither on the direction of propagation nor on retinal location.

The velocity was found independent of the kind of spreading induction. The energy of spreading induction suffers from decrement as it is propagated, but its velocity remains uniform. In these aspects the type of energy transmission of spreading induction is different from that of nervous conduction which follows the all-or-nothing law.

References

1) Granit, R., Erg. d. Physiol., 1950, **46**, 31.
2) Granit, R. & Svaetichin, G., Upsala Läk. för. Förh., 1939, **65**, 161.
3) Motokawa, K., J. Neurophysiol., 1949, **12**, 291.
4) Motokawa, K., J. Neurophysiol., 1949, **12**, 475.
5) Motokawa, K., J. Neurophysiol., 1950, **13**, 413.
6) Motokawa, K., J. Neurophysiol., 1951, **14**, 339.
7) Motokawa, K., Tohoku J. Exp. Med., 1951, **54**, 385.
8) Motokawa, K., Iwama, K. and Ebe, M., Jap. J. Physiol., 1952, **2**, 198.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

Experimental Study on the Disturbance of Acid-Base Balance during and after Bilateral Thoracotomy and on its Prevention

By

Togo Horiuchi

(堀内藤吾)

From the Surgical Clinic of Prof. S.-T. Katsura, Faculty of Medicine, Tohoku University, Sendai

(Received for publication, May 11, 1953)

Introduction

When we operated inside the thoracic cavity in old days, sometimes bilateral thoracotomy was forced to perform and the patient was confronted with death. In the earlier part of this century (1904), Sauerbruch, Brauer and others invented " Ueberdruck-Verfahren " (high pressure gas machine) which prevented the above mentioned danger. This apparatus became popular and a great progress in thoracic surgery was established. It has been, however, looked over for a long time that, when this apparatus is used, beside an advantage that the blood O_2 content increases, there occurs a great disadvantage that CO_2 accumulates in blood due to disturbance of expiration.[1)-6)] The first object of this study is to elucidate the condition to cause the accumulation of CO_2 and to clarify the mechanism of disturbance of acid-base balance encountered during and after the operation. To find out the counter measure to prevent such disturbance is the second object of this research.

Experimental

(I) The Disturbance of Acid-base Balance during and after Bilateral Thoracotomy with Constant Flowing of O_2 under Positive Pressure

Method

Rabbits and dogs were used for this experiment. In rabbits the chest wall was incised bilaterally, 3 to 4 cm. long under local anesthesia, and in dogs an incision of 20 cm. long was made also under local anesthesia, barbiturate having been administered previously. After thoracotomy, an air-tight tube was inserted in the trachea and was connected

to an apparatus of continuous O_2 flow under positive pressure (semi-closed system).

Chemical analysis of blood samples. Blood samples which were taken from A. femoralis with oiled syringe containing a small quantity of potassium oxalate sufficient to prevent coagulation were centrifuged to separate plasma. The plasma samples thus obtained were analysed as soon as possible. CO_2 content was measured by Van Slyke and Neill's method[7] with 0.5 cc. samples. The pH of the plasma was measured with Hasting and Sendroy's phenol red bicolor standard method[8]. The CO_2 tension, pCO_2 was read from Peters and Van Slyke's Nomogram[9] by the observed pH and the total plasma CO_2. When the data in question fell outside the scales of the nomogram, pCO_2 was calculated directly by Henderson-Hasserbalch's equation:

$$pCO_2=\frac{[\text{Total } CO_2]p}{0.1316\times 0.510\,(10^{pH-6.10}+1)}$$

Blood Hb (in g. per 100 cc.) was measured by Acid Hematin method (Sahli-Komiya).

$[HCO_3]$ corrected for pH 7.4, (mM/L. of plasma) was computed by the formula:[10][11]

$$\text{Hb mM/L.}=\frac{\text{Hb in g. per 100 cc.}\times 10}{16.7}$$

$$\beta=8.6+2.3\ (\text{Hb mM/L.})$$

$$\therefore\quad [HCO_3]p\ \text{cor.}=[HCO_3]p\ \text{obs.}+\beta\ (pH_s\ \text{obs.}-7.4)$$

Result

1) *The factors contributing to the accumulation of CO_2 during long standing opening of chest and constant flow of O_2 under positive pressure (semi-closed system)*

The CO_2 showed an ascending curve resembling hyperbola (See Table I). The CO_2 tension increased sharply making almost a straight line, while the pH values decreased also linearly. R_2 died 6 hours after opening the chest, when the pH dropped to 6.85 and the CO_2 tension ascended to 207 mm. Hg. As soon as the chest was opened, the respiratory rate was about 50 per minute, though the respiratory depth was not remarkably increased. The breathing got deeper with the lapse of time and the strained ventilation became gradually evident. 4 hours after operation the respiration decreased by and by. 5 hours later great spastic respiration appeared occurring about 20 times per minute and the pulse slowed down. The general condition got so worse that 6 hours later the heart stopped in diastole.

TABLE I

Changes in CO_2 during Long-timed Opening of Chest with Constant Flow of O_2 under Positive Pressure in Rabbits

Case Number	Item	Before thoracotomy	Time after start of thoracotomy					*	Remark	
			5 (min.)	1 (hour)	2 (hour)	5 (hour)	6 (hour)		Continuous press.	Intermitt. press
R_1	CO_2 vol %	35.9 (0.0)	52.6 (+16.7)	62.0 (+26.1)				58.1 (+22.1)	4 cm. H_2O	P=4/0 cm. H_2O R=40/Min.
R_2	CO_2 vol %	53.0 (0.0)	56.8 (+ 3.8)	69.2 (+16.2)	77.8 (+24.8)			59.9 (+16.9)	do	do
R_3	CO_2 vol %	49.1 (0.0)	57.3 (+ 8.2)	71.2 (+22.1)	75.4 (+26.3)			70.8 (+21.7)	do	do
R_5	CO_2 vol %	41.6 (0.0)	55.0 (+13.4)	54.5 (+12.9)	54.0 (+12.4)			56.5 (+14.9)	12 cm. H_2O	P=12/2 cm. H_2O R=40/Mim.
R_6	CO_2 vol %	38.5 (0.0)	41.6 (+ 3.1)	52.9 (+14.4)	69.3 (+30.8)			68.5 (+30.0)	do	do
R_7	CO_2 vol %	49.3 (0.0)		70.9 (+21.6)	82.5 (+33.2)	91.3 (+42.0)	92.0 (+42.7)			
	pH_s	7.32		7.28	7.18	7.00	6.85			
	pCO_2 mm. Hg.	41.5		66.0	94.8	155.2	207.1			
	HCO_3^- obs. mM/L.	21.3		29.8	34.4	36.4	34.1			
	Hb mM/L.	8.7		7.5	7.8	6.8	6.7			
	HCO_3^- cor. mM/L.	19.0		26.7	28.6	26.7	20.9		8 cm. H_2O	
	change in fixed acid mM/L.	–		−7.7	−9.6	−7.7	−1.9			
Average	CO_2 vol %	44.5 (0.0)	52.6 (+ 9.1)	63.5 (+19.1)	71.8 (+25.5)					

* The figures shown are the CO_2 vol % obtained when intermittent positive pressure was applied for 10 minutes after the bilateral thoracotomy was performed for 2 hours under constant flow of O_2 positive pressure (see Remark).

TABLE II

Changes in CO_2 Content, pH, pCO_2, Fixed Acid during and after Pleura Opened, in Dogs

Case Number	Item	Before thoracotomy	1 Hour after pleura opened	30 Min. after pleura closed	Time after end of operation	
					2 (day)	5 (day)
D_7	CO_2 vol %	56.5 (0.0)	57.6 (+ 1.1)		49.3 (− 7.2)	54.9 (− 1.6)
	pH_s	7.30 (0.0)	7.17 (− 0.13)		7.03 (− 0.29)	7.38 (+ 0.08)
	pCO_2 mm. Hg.	49.5 (0.0)	67.5 (+18.0)		71.5 (+22.0)	41.0 (− 8.5)
	HCO_3^- obs. mM/L.	27.5 (0.0)	28.0 (+ 0.5)		20.3 (− 7.2)	23.3 (− 4.2)
	Hb mM/L.	6.9 (0.0)	7.0 (+ 0.1)		9.6 (+ 2.7)	7.4 (+ 0.5)
	HCO_3^- cor. mM/L.	25.1 (0.0)	22.4 (− 2.7)		9.9 (−15.2)	18.2 (− 6.9)
	change in fixed acid mM/L.	−	+ 2.7		+15.2	+ 6.9
Average of D_1 D_2 D_3 D_4 D_5 D_6 & D_7	CO_2 vol %	47.9 (0.0)	52.0 (+ 4.5)	45.8 (− 0.7)	39.1 (−11.7)	
	pH_s	7.39 (0.0)	7.15 (− 0.24)	7.24 (− 0.16)	7.23 (− 0.13)	
	pCO_2 mm. Hg.	35.1 (0.0)	65.2 (+28.7)	46.3 (+13.6)	41.3 (+ 3.0)	
	HCO_3^- obs. mM/L.	21.5 (0.0)	22.5 (+ 1.0)	18.7 (− 0.9)	16.1 (− 8.8)	
	Hb mM/L.	7.0 (0.0)	7.1 (+ 0.1)	7.1 (+ 0.1)	7.2 (+ 0.4)	
	HCO_3^- cor. mM/L.	21.2 (0.0)	16.2 (− 5.0)	14.3 (− 5.1)	11.4 (−12.2)	
	change in fixed acid mM/L.	−	+ 5.0	+ 5.0	+12.2	

2) *Changes in CO_2 content, pH, CO_2 tension, concentration of bicarbonate and fixed acids during and after the opening of chest*

Table II shows the results of experiments on dogs. When both pleurae were opened during the course of operation, the CO_2 tension showed a tendency to rise and pH to fall as shown in Table I. This tendency was observed in the greatest degree in D_5; CO_2 tension increased to 47.9 mm. Hg. and pH fell 0.40 compared with the preoperative value. The average increase of pCO_2 was 28.7 mm. Hg. and decrease of pH was 0.24. Obviously this was due to respiratory acidosis. The change

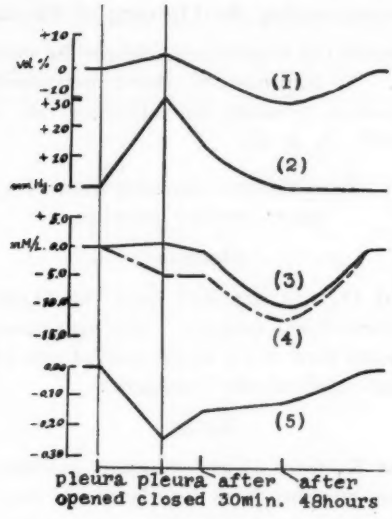


Fig. 1. Graph based on Table II shows the average changes of values of (1) total CO_2 (vol %), (2) pCO_2 mm. Hg., (3) $[HCO_3]$ mM/L., (4) $[HCO_3]$ corrected for pH, mM/L. and (5) pH_s. during and after operation.

of fixed acids was variable depending upon the individuals. In some increase and in others no change was found, the change being 5.0 mM/L. in average. Generally speaking, respiratory acidosis became complicated, more or less, with metabolic acidosis during the course of opening the chest. After the pleura was opened for an hour, the chest was closed by inflating the lungs sufficiently under about 20 cm. H_2O positive pressure. The CO_2 which increased during the course of operation returned to the preoperative value and the pH showed a tendency to increase 30 minutes after the closure of the pleura.

Concerning the postoperative course, all cases did not show any increase of pCO_2, except D_7 which suffered obviously from anoxic anoxia due to insufficient closure of the chest; no symptom indicating the respiratory acidosis was observed. In all cases, however, alkali reserve decreased remarkably (12.2 mM/L.) (metabolic acidosis). While pH in almost all cases were in the compensatory area, that in D_7 dropped to as low as 7.03 which was a dangerous area for the animal. It seems that this metabolic acidosis was due to postoperative anoxia which often produces a large amount of fixed acids by the disturbance in metabolism of the tissue cells.

(II) Prevention against the Respiratory Acidosis Accompanying the Opening of Thorax

In order to prevent the respiratory acidosis the ventilation of alveolar gas was considered. To improve the above mentioned constant flowing procedure under positive pressure the following two modifications were studied experimentally (A & B).

(A) Intermittent administration of O_2 under positive pressure

Method

The compressed O_2 was supplied from the O_2-bomb to a positive pressure machine (semi-closed system). The apparatus was regulated so as to allow the constant flow of O_2 at the rate of one liter per 30 seconds, and the pressure was rhythmically changed.

Result

Rabbits R_8 and R_9 were observed under positive pressure of 8 cm. H_2O. The hydrolic pressure was regulated to synchronize with the natural rythm of respiration. While the pressure in the alveolar cavity fluctuated between 8 cm. H_2O in inspirium and 4 cm. H_2O in expirium, the accumulation of CO_2 was hardly prevented. R_{10} was experimented, so that the rate of respiration was 20 per minute and pressure swinged between 8 cm. H_2O and 0 cm. H_2O. The blood CO_2 decreased, but the result was not yet satisfactory. R_{11} and R_{12} were conditioned to the rate of respiration of 20 per minute, and the pressure between 10 cm. H_2O and 0 cm. H_2O. The CO_2 got lower than the preoperative level, showing that the condition was satisfactory. R_{13} and R_{14} were examined for acid-base balance under the same condition. The decrease of CO_2 accompanied with the fall of pCO_2; the respiratory acidosis was completely under control. But I have to point out that there was a tendency of an increase of fixed acid, and a slight fall of pH during the course of this method (see Fig. 2 (A)). Judging from the recovery of the metabolic acidosis after the closure of pleura, this phenomenon seems to be related to this mode of artificial respiration. It may have been resulted from the production of ketone substances due to hyperventilation.

(B) Constant flowing under positive pressure by intermittent insufflation of excessive O_2

Method

Both pleural cavities were opened under certain positive pressures (P.) and insufflated with O_2 at a constant speed of flowing (Insufflation

TABLE III

Changes in CO_2 when the Intermittent Administration of O_2 under Positive Pressure was used on Various Conditions in Rabbits

Case number	Item	Before thoracotomy	After pleura-opening: 30 (min.)	After pleura-opening: 1 (hour)	After pleura-opening: 2 (hour)	1 Hour after pleura-closing	Remark: Intermitt. pressure	Remark: Rate of respiration
R_8	CO_2 vol %	41.2	53.8	58.4	65.5	46.0	P=8/4 cm. H_2O	R=40
R_9	CO_2 vol %	45.3	56.5	66.0	63.7	50.0	do	do
R_{10}	CO_2 vol %	50.2	53.3	57.7	61.8		P=8/0 cm. H_2O	R=20
R_{11}	CO_2 vol %	51.0	44.1	40.8	40.4	51.3	P=10/0 cm.H_2O	R=20
R_{12}	CO_2 vol %	35.0	30.6	32.7	34.4	45.6	do	do
	CO_2 vol %	44.4	40.3	34.9		51.6		
	pH_s	7.33	7.30	7.28		7.25		
	pCO_2 mm. Hg.	36.5	35.0	32.1		51.0		
R_{13}	HCO_3^- obs. mM/L.	18.8	17.1	14.6		21.5	do	do
	Hb mM/L.	6.9	6.9	7.5		6.6		
	HCO_3^- cor. mM/L.	17.1	14.7	11.5		17.9		
	change in fixed acid mM/L.	—	+2.4	+5.6		−0.8		
	CO_2 vol %	55.8	43.7	32.3		38.4		
	pH_s	7.30	7.27	7.28		7.25		
	pCO_2 mm. Hg.	49.5	41.7	30.0		37.8		
R_{14}	HCO_3^- obs. mM/L.	23.6	18.5	13.6		16.2	do	do
	Hb mM/L.	7.5	7.2	7.2		7.2		
	HCO_3^- cor. mM/L.	21.0	15.2	10.6		12.4		
	change in fixed acid mM/L.	—	+5.8	+10.4		+8.6		
	CO_2 vol %	50.1	42.0	33.6		45.0		
Average of R_{13} & R_{14}	pH_s	7.32	7.29	7.28		7.25		
	pCO_2 mm. Hg.	43.0	38.4	31.1		44.4		
	HCO_3^- obs. mM/L.	21.2	17.8	14.1		18.9	do	do
	Hb mM/L.	7.2	7.1	7.4		6.9		
	HCO_3^- cor. mM/L.	19.1	15.0	11.1		15.2		
	change in fixed acid mM/L.	—	+4.1	+8.0		+3.9		

of O_2 at the rate of 1 liter per 30 seconds from the O_2-bomb to the positive pressure machine). Then, during the course of opening of chest, a certain amount of O_2 (5 liters) was insufflated at a high speed (at the rate of one liter per 4 seconds) intermittently (frequency F.). Subsequently the positive pressure rose by 2 cm. H_2O higher than given positive pressure

TABLE

Changes in CO_2 when Constant Flowing under Excessive O_2 was used on

Case number	Item	Before thoracotomy	After pleura-opening		
			30 (min.)	1 (hour)	2. (hour)
R_{16}	CO_2 vol %	34.5	42.0	51.5	55.8
R_{17}	CO_2 vol %	47.6	57.6	56.8	62.7
R_{20}	CO_2 vol %	29.6	29.0	27.6	29.4
R_{21}	CO_2 vol %	48.9	44.0	44.8	44.6
	CO_2 vol %	60.2	59.8	59.2	
	pH_s	7.33	7.33	7.35	
	pCO_2 mm. Hg.	49.9	49.0	46.2	
R_{22}	HCO_3^- obs. mM/L.	25.3	25.1	25.0	
	Hb mM/L.	8.1	8.5	8.5	
	HCO_3^- cor. mM/L.	23.4	23.1	23.6	
	change in fixed acid mM/L.	—	+ 0.3	− 0.2	
	CO_2 vol %	43.8	43.8	39.7	
	pH_s	7.33	7.33	7.30	
	pCO_2 mm. Hg.	36.0	36.0	35.0	
R_{23}	HCO_2^- obs. mM/L.	18.5	18.5	16.8	
	Hb mM/L.	7.2	7.2	7.5	
	HCO_3^- cor. mM/L.	16.7	16.7	14.3	
	change in fixed acid mM/L.	—	0.0	+ 2.4	
	Co_2 vol %	52.0	51.8	44.5	
	pH_s	7.33	7.33	7.33	
Average of R_{22} & R_{23}	pCO_2 mm. Hg.	43.0	44.5	40.6	
	HCO_3^- obs. mM/L.	21.9	21.8	20.9	
	Hb mM/L.	7.7	7.9	8.0	
	HCO_3^- cor. mM/L.	20.1	19.9	19.0	
	change in fixed acid mM/L.	—	+ 0.2	+ 1.1	

followed by the expansion of lungs.

Result

R_{16} was under the condition of P.=4.0 cm. H_2O, F.=one time per 15 minutes. The increase of CO_2 was lower than that observed in the constant flowing under positive pressure (Table I), but the removal of CO_2 was not yet satisfactory. R_{17} was under the condition of P.=6 cm. H_2O, F.=2 times per 5 minutes. The results were also not good. R_{20}

IV

Positive Pressure by Intermittent Insufflation of Various Conditions in Rabbits

After pleura-closing			Remark	
1 (hour)	2 (day)	3 (day)	Positive press.	Frequency
			P=4 cm. H_2O	F=1/15 Min.
80.7			P=6 cm. H_2O	F=2/5 Min.
41.9			P=4 cm. H_2O	F=2/5 Min.
50.9			do	do
62.0		59.9		
7.33		7.42		
51.8		42.7		
26.1		25.4	do	do
8.5		6.8		
24.1		25.4		
− 0.7		− 2.0		
44.0	46.8			
7.28	7.35			
41.0	36.3			
18.5	19.7		do	do
7.5	6.5			
15.4	18.5			
+ 1.3	− 1.8			
53.0				
7.31				
46.4				
22.3			do	do
8.0				
19.8				
+ 0.3				

and R_{21} were under the condition of P.=4 cm. H_2O, F.=2 times per 5 minutes. The CO_2 did not increase and satisfactory results were thus obtained. R_{22} and R_{23} were examined for acid-base balance under the same condition. The pCO_2, pH and alkali reserve did not show significant changes. Therefore, the respiratory acidosis was to be considered completely prevented, not causing also the metabolic acidosis. An ideal condition was found (see Fig. 2 (B)). Furthermore, R_{22} was killed 10 days after the operation, and its lungs were examined histologically. No

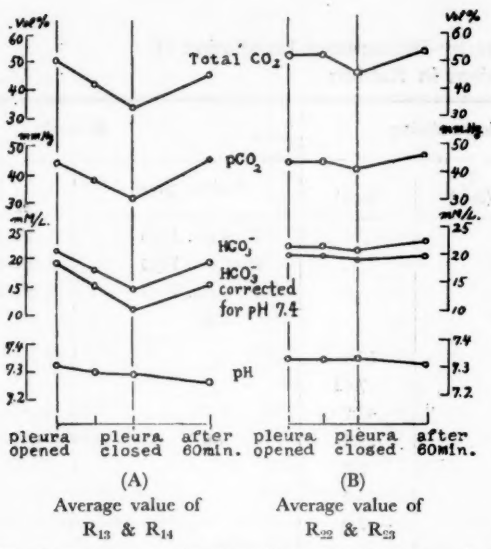


Fig. 2. Comparison of (A) intermittent administration of O_2 under positive pressure & (B) constant flowing under positive pressure by intermittent insufflation of excessive O_2. Experiments on rabbits.

pathologic processes were detected.

Comment

Little significance has been given clinically to the respiratory acidosis. This fact may be explained as follows: the respiratory acidosis in diseases treated ordinarily in clinic is almost always accompanied with anoxia, because the rate at which CO_2 diffuses through animal membranes is 20 to 30 times as great as the rate at which O_2 diffuses (Krogh),[11] in any individual breathing the usual atmosphere, interference with the gas exchange results in death from O_2 want before CO_2 accumulation becomes dangerous[11)-13)]. When both pleurae of animals are opened under positive pressure of O_2, a peculiar condition is recognized. That is, anoxia doesn't appear because of sufficient supply of O_2, but the accumulation of CO_2 develops severe respiratory acidosis and the animal is exposed to death. In clinical cases, however, this kind of acidosis develops usually so gradually, that it will be followed at first by a shift of the CO_2 absorption curve, then by a change in the concentration of plasma bicarbonate. Both of them are the compensatory mechanism and bring the pH which has fallen for a while back to within the normal range. Whereas the respiratory acidosis which will occur during thoracotomy has not enough time to

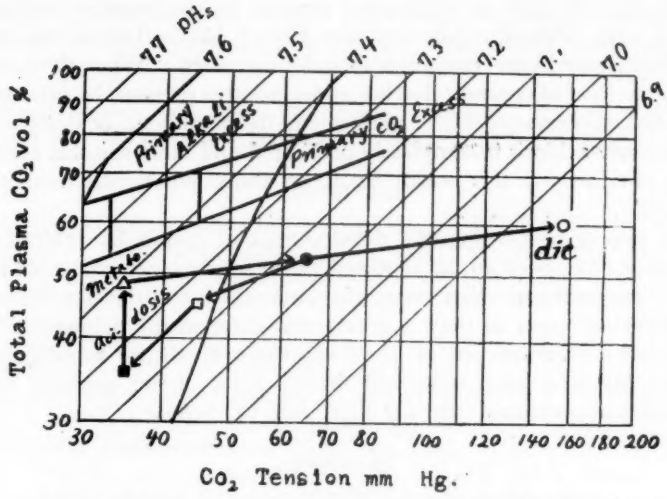


Fig. 3. The changes of acid-base balance during and after bilateral thoracotomy drawn in a logarithmic chart after Peters based on the data of table I and table II.

△—Values preoperative and after complete recovery.

●—Values during bilaterally open chest.

○—Values of cases of death from severe respiratory acidosis caused by long standing open chest.

□—Values immediately after closure of chest.

■—Values a few days after operation.

be alleviated by compensatory mechanism, the respiratory acidosis develops rather acutely along with the shift of the CO_2 absorption curve. Consequently, the pH drops sharply and the circulatory collapse becomes imminent (see Fig. 3). The respiratory acidosis during the opening of chest under the special condition of using O_2 flow under positive pressure, is characterized by the lack of tachypnea and cyanosis which are the sole symptoms of respiratory acidosis ordinarily observed in clinic. Therefore, a danger of acidosis would be doubled. A method to prevent its occurrence *during* the operation should be considered important.

A method of intermittent administration of O_2 under positive pressure following the similar principle of Crafood[5] was performed, and the accumulation of CO_2 was successfully prevented. Though the prevention of respiratory acidosis is possible while the prevention of metabolic acidosis is impossible by such a procedure, it seems judging from the patterns of acid-base balance, that the hyperventilation will be followed by a metabolic acidosis due to overproduction of ketone substances or the increased fixed

acid by the overwork of respiratory muscle, as the artificial respiration interfers with normal respiration (see Fig. 2 (A)). This is the reason why unnecessary hyperventilation should be avoided. When thoracotomy with the method of constant flowing under positive pressure by intermittent insufflation of excessive O_2 is performed, the metabolic acidosis as well as respiratory acidosis is completely prevented and more suitable condition can be obtained by this means than the above mentioned method (see Fig. 2 (B)).

No papers concerning the development of metabolic acidosis postoperatively have been so far reported as far as they are accessible to me. Anemic, stagnant and other types of anoxia which can develop frequently after operation seem to play a great role in metabolic acidosis and if it complicates with anoxic anoxia the danger of acidosis will be exaggerated. The prevention of such untoward factors is an urgent problem besides the usual postoperative care for fluid and O_2 supply.

Summary

An experimental study on disturbances of acid-base balance encountered during operations of opened chest was carried out in dogs and rabbits. Special attention was given to the effects of methods for continuos O_2 supply under positive pressure. The following results were obtained:

1. When bilateral thoracotomy is performed with constant flowing of O_2 under positive pressure, the removal of CO_2 is hindered, the respiratory acidosis sets in with the lapse of time and the danger ensues. In fact, in spite of enough supply of O_2, the animal dies due to the acidosis.
2. The respiratory acidosis during operation will be relieved by closing the chest.
3. The respiratory acidosis during operation is often complicated by metabolic acidosis.
4. Metabolic acidosis appears few days after operation and it tends to increase.

Concerning the research how to prevent the occurrence of acidosis during operation the following results were obtained:

5. When the intermittent administration of O_2 under positive pressure or the constant flowing under positive pressure by intermittent insufflation of excessive O_2 is used to expedite the exchange of the alveolar gas, the respiratory acidosis is prevented.
6. The partial pressure of CO_2 is not increased by the type of intermittent administration of O_2 under positive pressure, but the alkali reserve decreases and the pH falls.
7. Metabolic acidosis as well as respiratory acidosis do not occur by the type of constant flowing under positive pressure by intermittent

insufflation of excessive O_2. More physiologic, accordingly more suitable condition can be obtained by this means than by the above mentioned method.

Kind advices were given by Prof. K. Matsuda, of applied physiology I should like to express my gratitude to him.

References

1) Yugaya, Nihon Geka Hokan (Arch. Jap. Chir.), 1930, **7**, 1.
2) Suguru, Nihon Geka Hokan (Arch. Jap. Chir.), 1933, **10**, 512.
3) Yugaya, Nihon Geka Hokan (Arch. Jap. Chir.), 1928, **5**, 769.
4) Sekiguchi & Uebayashi, Nihon Geka Gakkai Zasshi, 1926–27, **27**, 904.
5) Crafood, J. Thorac. Surg., 1940, **9**, 237.
6) Mautz, J. Thorac. Sug., 1941, **10**, 544.
7) Van Slyke & Neill, J. Biol. Chem., 1924, **61**, 523.
8) Hasting & Sendroy, J. Biol. Chem., 1924, **61**, 695.
9) Van Slyke & Sendroy, J. Biol. Chem., 1928, **79**, 781.
10) Gibbon, Allbritten, Stayman & Judd, Ann. Surg., 1950, **132**, 611.
11) Peters & Van Slyke, Quantitative Clinical Chemistry, Vol. 1, Baltimore, 1937.
12) Cantarow, Clinical Biochemistry, 4th Ed., Phil. & London, 1949.
13) Hasting, Neill, Morgan & Binger, J. Clin. Invest., 1924, **1**, 25.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

On Motor and Sensory Nerve Systems in in Lumber Cord in Man

By

Mamoru Suzuki
(鈴木 衛)

From the Anatomical Laboratory Of Prof. H. Seto,
Tohoku University, Sendai

(Received for publication, May 14, 1953)

There have been innumerable histological studies reported on the subject of spinal cord, using general staining methods. However, little has been done under utilization of special staining methods applied to nerve fibrils, which should form the basis of final decision in problems concerning nerve tissues. And even these few studies had only old impregnation methods of rather dubious efficacy to rely upon, so that it may be said that many a problem is as yet left unsolved. In consideration of such a situation, I undertook a new study on the subject using special staining method, and collating my observations with those of the preceding researchers, tried to contribute whatever little I could to the elucidation of the problems.

As material, I chose lumbar cords of human infants and adults fixed in 10% neutral formol for a long period, cut them into series of cross and sagittal frozen sections, and having stained them with Seto's silver impregnation, prepared a large number of very beautiful preparations, which I subjected to minute scrutiny.

Individual Observations

Motor nerve cells in lumbar cord: Studies on the motor nerve cells in the spinal cord utilizing special staining methods were undertaken by Golgi,[1] Cajal,[2] van Gehuchten,[3] Lenhossék,[4] Lavdowsky,[5] Obersteiner,[6] Kölliker,[7] Ziehen,[8] Bielshowsky,[9] Edinger[10] and others at the turn of the present century, and some results were achieved, but owing to the imperfect means of study under their command, and also due to the influence of neuron theory of Langley, their observations seem to contain not a few questionable assertions. To my surprise, I found few reports, if any, on the subject utilizing modern silver methods made available since those days.

As the consequence, some of the following observations I made with

the ideally impregnated preparations were new, unprecedented in the literature on the subject as far as I know. I will take up the matter of motor nerve cells in the spinal cord in the first place. In all previous reports, these cells were represented as existing only in groups in cornu ventrale, but in my specimens, I found some of them, in sporadic small number, also in pars intermedia (Fig. 1), in the lateral side of cornu dorsale (Fig. 2) and in the medial parts (Fig. 3) as well as in the basis thereof. These cells, I need not emphasize, are typical motor cells of the peculiar shape as described hereunder.

Fig. 1

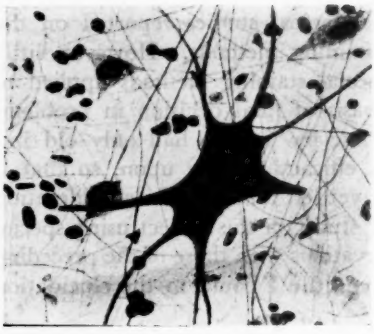


Fig. 2

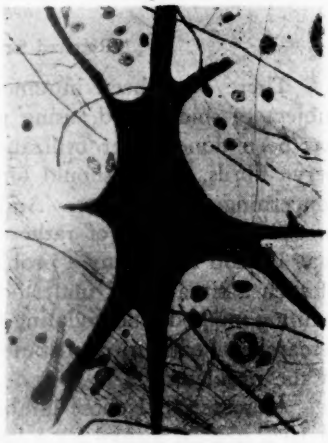


Fig. 1. A motor nerve cell and a few vegetative cells seen in the medial side of pars intermedia of the cranial lumbar cord in human infant. Thin fibres are vegetative, thick ones are chiefly sensory. Cross section. Seto's impregnation. X 600, reduced to 1/2.

Fig. 2. A motor cell seen in the lateral side of cornu dorsale of the caudal lumbar cord in human infant. Cross section. Same staining. X 600, reduced to 1/2.

The motor nerve cells in the lumbar cord are clearly distinguishable from the undermentioned sensory and vegetative cells, in that they are far larger in size than the latter two, show concave contour and send out many stout processes, in short, have the appearance of rugged virility. Motor cells of such peculiar conformation have been recently proven by Sato[11] and Atsuki[12] in this laboratory, in the nucleus n. hypoglossi and the nucleus n. oculomotorii. Observations on further details concerning these motor cells are described in the following.

The motor cells in cornu ventrale (Fig. 4) are more decidedly typical

Fig. 3

Fig. 4

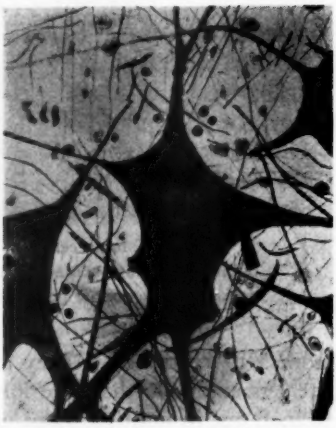


Fig. 3. A motor cell and a few vegetative cells seen in the medial side of cornu dorsale of lumbar swelling in human infant. Cross section. Same staining. X 600, reduced to 2/3.

Fig. 4. Typical motor cells in the lateral middle cell group in cornu ventrale of lumbar swelling. Thick fibres are motor and sensory fibres, thin fibres are vegetative fibres. Cross section. Same staining. X 600, reduced to 1/2.

in appearance than those in pars intermedia and cornu dorsale. A rather small, round or oval, nucleus is situated chiefly at the center of the cell body. The neuro-fibrils show a minute reticular arrangement in the cell body, but upon emerging into the processes, they appear compressed and elongated. The long process branches out at a right angle to the long axis of the cells which run parallel to the length of the spinal cord. Their incipient part is much thinner than that of the short processes, but not far from the cell body, myelin is formed around it and it becomes stout and strongly stainable.

Generally speaking, one long process is sent out by each motor cell, but the total number of the stout motor root fibres in any given cross section is larger by 20% than that of the motor cells in which they originate. Such an excess of the number of long processes has been observed also in the medulla oblongata and mesencephalon by Sato[11] and Atsuki.[12] According to them, this phenomenon is due to the fact that some motor cells send out two long processes each, and that some processes branch out into 2 rami. I could not observe such duplications in my specimens, but I consent with them in their interpretation.

The short processes of the motor cells are very peculiar in comparison with those of other nerve cells. The number is rather large, ranging

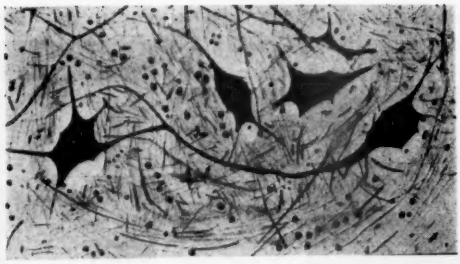


Fig. 5. Motor cells in the lateral middle group in cornu ventrale of lumbar swelling. Human infant. A true anastomosis of short processes between both motor cells is seen. Cross section. Same staining. X 200, reduced to 1/2.

from 5 to 7 per cell. They are stout in size and run a long course, but are less stainable than the long processes. Most of these short processes branch out in multiple rami upon emerging from the cell body, but some of them end without branching. The great length of the short processes of motor cells seems to be fraught with some deep importance. I regret I could not make a full study on the terminal formation of the end branches of the short processes, but I was led to believe that they end sharply in free endings. What is of some interest is the fact that there exist some ture anastomosis between short processes of different motor cells, as shown in Fig. 5.

Waldeyer,[13] Obersteiner[6] and Ziehen[8] have classified the motor cell groups in cornu ventrale into one medial group and three lateral groups, and Jacobsohn[14] into one each medial and lateral groups, the former again into ventral and dorsal subgroups and the latter into ventral, middle and dorsal subgroups. My observations accorded with Jacobsohn's in general outline, the medial group is composed of cells much smaller in number and size than those in the lateral group. The medial group is situated on the medial periphery of cornu ventrale, and can be subdivided into ventral and dorsal subgroups. The development of this medial group is very weak, throughout the whole length of the lumbar cord, the number of cells diminishing further in the caudal parts, especially in the medio-dorsal subgroup, so that they do not necessarily appear on each cross section. The medial ventral subgroup appears on most cross sections, but sometimes the number of the cells is reduced to 1 or 2.

The lateral group, on the other hand, shows a very marked development, and in the higher and lower parts of lumbar cord, it can be subdivided into the ventral, middle and dorsal subgroups. However, in the lumbar swelling, the number of the cells increases further, and it seems a division into a larger number of subgroups is justified. This

increased number indicates of course the originating of many motor nerve fibres to the lower limbs.

As shown above, I could observe existence of motor cells also in other parts of the lumbar cord than in the ventral horn, which fact has scarcely been noticed in any previous reports. Stöhr[15] says in his histology (1951) that the existence of motor cells in the spinal cord is limited to cornu ventrale and pars intermedia, Obersteiner[6] remarks that they are found in his so-called reticular process i.e. formatio reticularis in rare cases, and the above two statements exhaust all what I could find in the whole literature on the subject, touching the problem.

According to my exhaustive study, the motor cells in pars intermedia are represented in sporadic existence, especially in the upper part of the lumbar cord. The area is delimited to the medial side of pars intermedia and the number to 3 or 4 at the maximum. These cells belong to the motor system morphologically, particularly resembling the small motor cells found in the medial group in cornu ventrale (Fig. 1).

I could not observe motor cells in the formatio reticularis, as Obersteiner[6] asserts, but on the dorso-medial side thereof, that is, on the lateral side of cornu dorsale (Fig. 2) and its medial side, and even in its basis I found not rarely the existence of a few virile looking motor cells. These cells also are similar to the small motor cells in the medial group of cornu ventrale. But I regret to say that I could not clarify the course of the long processes going out of these cells.

Plexus in motor cell groups in cornu ventrale: the motor cells are buried in the plexus of incoming nerve fibres formed in their cell groups. It may be inferred that this plexus is formed of many kinds of incoming fibres, like in the various motor nuclei of brain stem, such as effernt fibres from the pyramidal and extrapyramidal tracts, reflex sensory fibres from the dorsal roots of the spinal cord, and sympathetic and parasympathetic nerve fibres. According to my observations, the motor fibres come in chiefly through the tractus corticospinalis lateralis, and the reflex sensory fibres through the entrance zone of the dorsal root fibres, in dispersed formations, penetrate the medial parts of substantia gelatinosa of Rolando, cornu dorsale and pars intermedia, to reach into motor cell groups in cornu ventrale. Minute vegetative fibres also come in from the surroundings of the motor cell groups.

All the above mentioned incoming fibres are far thinner than the short processes of motor cells, so that the histological distinction between them is very clear. Among the incoming fibres, those of sensory and motor nature are stouter and more strongly staining, than the vegetative fibres, and the distinction between them is also very easy. However, I found the terminations of these incoming fibres around the motor cells

are never represented by pericellular reticular terminations, or the so-called annular or pin-head corpuscles, as has been assumed hitherto.

As shown in the illustration, few, if any, terminal or reticular formations are seen, but incoming fibres of various sizes are seen running intertwining at a considerable space therefrom. However, it may be presumed that the incoming motor and sensory fibres form specific endings, which probably are represented by very simple branched terminations among the short processes, rather than around the cell bodies.

In studying the physiological meaning of motor cells in spinal cord. it may be assumed that they do not function unless externally stimulated, For this reason, the short processes receptive of stimuli are particularly strongly developed and are seen running far into the maze of incoming fibres of every description. The transmission of stimuli to these motor cells is presumably accomplished, as in the cerebellar cortex (Yamamoto),[16] in nucleus n. hypoglossi (Sato)[11] and nucleus n. oculomotorii (Atsuki),[12] by that the stimuli from the incoming fibres excite the neuroglial cells, which probably produce endocrinous substances, which in turn stimulate the motor cells, in particular, their short processes, and this excitement of the nerve cells is transmitted finally to the periphery through their long processes. So, the transmission of stimuli to the spinal motor cells is not represented by the fatal relation of one incoming fibre and one nerve cell, as the hitherto generally accepted neuron theory by Langley would premise.

Studies on the sensory nerve cells in the spinal cord under utilization of general staining methods, especially Nissl's staining, have been reported by Edinger,[10] Déjérine[17] and Ranson,[18] and on the classification of sensory cell groups under utilization of general staining methods by Jacobsohn,[14] Lohnstamm[19] and Massaza.[20] However, as methods specially adapted to the study have not been used, the morphological observations on these sensory cells must be said to contain many defects.

Jacobsohn[14] has named the small-sized cell group found outside the medial side of substantia gelatinosa " nucleus proprius sensibilis," the cell group found around the above nucleus and arranged between zona spongiosa and the medial side of substantia gelatinosa " nucleus magnocellularis pericornualis " and the cell group in the head of cornu dorsale " nucleus magnocellularis pericornualis centralis." Ziehen[8] observed in his so-called Zonalschicht or zona spongiosa some medium-sized triangular or pyramidal cells. Kuru[21] assumed through experiment the existence of nerve cells transmitting senses of pain and warmth in zona spongiosa, and identified them with the posteromarginal cells of Bok[22] and marginal cells of Waldeyer.[16] Ziehen[8] named the nerve cells in the head of cornu dorsale Innenzellen, Wldeyer[13] called the group of them " nucleus cornu

dorsalis" and Bok, "nucleus proprius cornu posterioris." Kuru,[21] by way of experiment, arrived at the opinion that sensory cells for transmission of tactile sense are contained in this cell group. The Clarke's cell group in the medial basis of cornu dorsale is known to be of sensory nature. Kuru is said to have proven experimentally that sensory cells responding to sexual sensation exist in the dorso-lateral side of pars intermedia not only of the sacral but also of the lumbar cord.

Sensory cells in my impregnated lumar cord stand between the above mentioned motor cells and the undermentioned vegetative cells in size and are clearly distinct from the two latter in form. Above all, it must be specially mentioned that, in spite of the fact that the sensory cells in the sensory nuclei in the brain stem show fat, rounded feminine appearance, as described by Atsuki[12] recently, those in spinal cord show such a feminine outline in only comparatively rare cases, being generally elongated spindle or triangle in form. Especially almost all of the sensory cells existing in the head of cornu dorsale, substantia gelatinosa and zona spongiosa are of oblong shape.

Not only the long processes, but also the short ones of the sensory cells give the appearance of stoutness by their strong silver affinity, but the number of the short processes is smaller than that of the motor cells, their size is smaller and their length is shorter. These short processes branch out in 2 or 3 rami, but sometimes without branching, generally to end sharply.

It is of some interest that the shape and size of the sensory cells in the lumbar cord are more or less different according to their localisation. For example, the sensory cells found in zona spongiosa (Fig. 6) are all oblong spindle-shaped and are arranged in 1 or 2 rows along the circumference of substantia gelatinosa. The cell nucleus is oval and centrally situated in general, and take up only about 1/5 of the entire area of the cell body. Their short processes are few in number and rather short in length, generally ending in zona spongiosa, but some extend into substantia gelatinosa or into zona marginalis. The long processes run along the medial or lateral edges of substantia gelatinosa. I could not observe long processes branching out of the short, as Ziehen[8] will have observed.

In the substantia gelatinosa, there are sensory cells on both the ventral and dorsal sides. Those on the dorsal side (Fig. 7) are extremely small in number, only 2 or 3, and even sometimes not one being seen on a cross section. They are spindle or star-shaped but somewhat stout, with a round or oval cell nucleus centrally located, and with weak short processes ending generally sharply. The long processes are assumed to be ascendent, like those of the sensory cells on the ventral side.

In the ventral side there are seen 4 or 5 of sensory cells usually on

Fig. 6

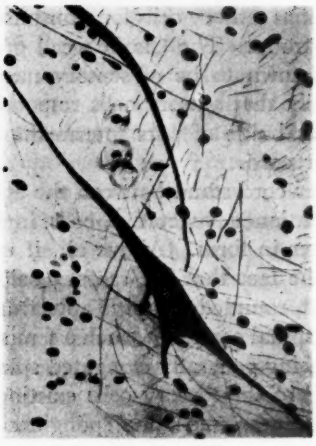


Fig. 7

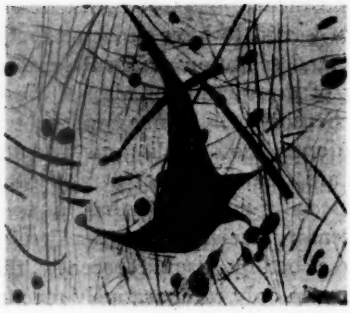


Fig. 6. A elongated spindleshaped sensory cell seen in zona spongiosa of lumbar swelling in human infant. Cross section. Same staining. X 600, reduced to 1/2.

Fig. 7. A fat star-shaped senory cell seen in the dorsal side of substantia gelatinosa of Rolando of lumbar swelling. Human infant. Short processes are remarkably short. Cross section. Same staining. X 600, reduced to 1/2.

one cross section, which are of elongated spindle shape, with the both ends oriented ventrally and dorsally, and with a oval cell nucleus centrally placed, as shwon in Figs. 8 and 9. The short processes are strongly stainable, stout and generally longer than those in the two kinds of sensory cells described above, sometimes reaching outside the substantia gelationsa. They divide into 2 or 3 rami or unbranched, always ending sharply. The long process is rather thin at the beginning but gradually grows thicker and stouter and takes chiefly ascending course, as may be readily seen from the sagittal section (Fig. 10). It sometimes shows branching out in two.

Sano[23] has observed small nerve cells in the gelatinous substance and concluded that this substance represented a vegetative nerve center. I of course concur in his opinion, since the middle area of substantia gelatinosa consists entirely of small nerve cells, but they are utterly different morphologically from the sensory cells found in the dorsal and ventral sides of substantia gelatinosa. Jacobsohn[14] concludes that the small nerve cells in this substance are of sensory nature, but I need not point out the error of his assertion.

In the cornu dorsale also exist some sporadic nerve cells belonging

Fig. 8

Fig. 9

Fig. 8. A small elongated spindle-shaped sensory cell and a few vegetative cells found in the dorsal side of substantia gelatinosa of lumbar swelling. Cross section. Same staining. X 600, reduced to 1/2.

Fig. 9. A larger spindle-shaped sensory cell found in the dorsal side of substantia gelatinosa of lubmar swelling. Human infant. 1 long process. Cross section. Same staining. X 600, reduced to 2/3.

to the category of sensory cells. It may have been these that Bok[22] has called "nucleus proprius cornu posterioris" and Ziehen[8] has called Innenzellen. The shape of these cells is variable, sometimes broad spindle-shaped, sometimes oblong spindle-shaped, sometimes triangular and sometimes round. The broad spindleshaped cells (Fig. 11) are rather numerous toward the medial side of the head of cornu dorsale, are provided with round or oval cell nucleus situated approximately on the center, and send out 4–6 short processes. The oblong spindle-shaped or triangular cells (Fig. 12) are chiefly located in the outer side of the head of corn dorsale, are smaller in size and number than the above and are generally provided with 3–4 short processes. The round cells (Fig. 13) are much rarer than the two above, found only in the head and middle parts of cornu dorsale, and are morphologically very similar to the sensory cells in the sensory nuclei in the brain stem. They are very stainable and their short processes are ill-developed and small in number.

Nucleus doralis of Clarke is very well developed in the thoracal cord, especially in its lower parts, the nerve cells numbering from 20 to 30 in one cross section, but in the lumbar cord, their number diminishes rapidly, numbering only from 3 to 7 per cross section, diminishing further as the

Fig. 10

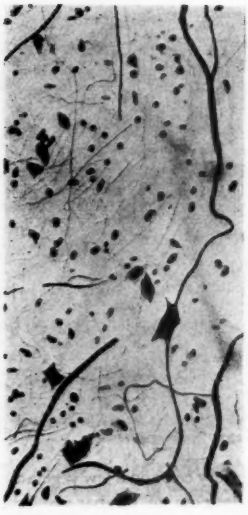


Fig. 11

Fig. 10. A sensory cell provided with a ascending long process dividing into 2 rami seen in the ventral side of substantia gelatinosa of the cranial lumbar cord. Human adult. Sagittal section. Thick fibres are sensory, thin fibres are vegetative. Same staining. X 200, reduced to 2/45.

Fig. 11. A rather large broad spindle-shaped sensory cell found in the medial side of the head of cornu dorsale of lumbar swelling. Human infant. Cross section. Same staining. X 600, reduced to 2/3.

caudal parts are approached. The localisation of this nucleus in the lumbar cord is shifted more dorsalwards than in the thoracal cord where the nerve cells are seen in the ventro-medial side of cornu dorsale, that is, to the medial side of the middle part of cornu dorsale.

These nerve cells are perhaps the second largest in the spinal cord, next only to the motor cells, being provided with 2–5 stout short processes, and give the appearance as if they were motor cells, but as shown in Fig. 14, their contour is rounded and feminine and show a similarity in form to the sensory cells in the sensory nuclei in the brain stem. Their short processes are also different from those of motor cells, being stout but branching out before long, and never running a long course to end sharply. Consequently these are concluded to be sensory and not motor in nature. Ziehen[8] says he found stellar cells in the central parts of the Clarke's nucleus and spindle-shaped cells in its marginal parts, but I could not observe any such views.

The long processes of these cells emerge from the cells on the ventral side and describing an arc laterally, run to the tractus spino-cerebellaris

Fig. 12

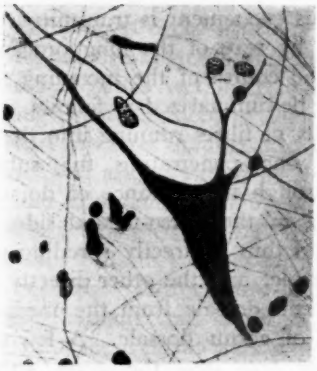


Fig. 13

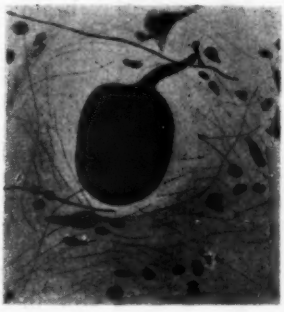


Fig. 12. A small triangular sensory cell found in the lateral side of the head of cornu dorsale of lumbar swelling. Human infant. Cross section. Same staining. X 600, reduced to 2/3.

Fig. 13. A round sensory cell and a few vegetative cells seen in the middle part of cornu dorsale. Human infant. Cross section. Same staining. X 600, reduced to 2/3.

lateralis of the same side. Obersteiner[6] asserts that he has observed in the Clarke's nucleus such nerve cells provided with 2 long processes running rostral- and caudalwards each, but in my sagittal sections I could observe none of the sort. Their cell nucleus is round or oval and placed excentrically.

Kuru[21] observed a group of sensory cells in the sacral cord close to the dorsal side of cornu laterale, which show retrogressive degeneration upon dissection of fasciculus ventralis, and named it "nucleus intermediodorsolateralis." He assumed that it was connected with the sexual sense. In his recent studies, he has found such cell groups also in the thoracal and lumbar cords, but in my researches, I could not set the existence of such a group in the lumbar cord beyond doubt, though I could observe it in the sacral cord.

Sensory cells in the lumbar cord, together with their short processes are buried in a complicated plexus. This plexus consists of incoming sensory fibres originating in the spinal ganglia and vegetative fibres. The latter are minute and weakly stainable and seem to end in reticular arrangement, while the former are stout and strongly stainable, but I could not clarify their terminal formation. I only presume that they terminate in simple branched endings. Stimuli are transmitted, as in the case of motor cells, from the incoming sensory fibres into the glial cells probably of endocrinous nature, which in turn secondarily excite the sensory cells

Fig. 14. Nerve cells in nucleus dorsale in cranial lumbar cord in human infant. Nerve cells show a rounded and feminine contour, provided with a few short processes. Cross section. Same staining. X 600, reduced to 1/2.

and their short processes through their secretion, and this excitement is transmitted to the brain center by way of the long processes.

As for the course of the incoming sensory fibres, it may be summarized as follows. There are two kinds of fibres running into the cornu dorsale—the one penetrating the substantia gelatinosa through the entrance of dorsal root fibres formed in the dorso-medial side of this substance, and thus indirectly spreding out into cornu dorsale, and the other directly penetrating the cornu dorsale from the medial side of the head of cornu dorsale. A few sensory fibres directly run into the zona spongiosa through the entrance of dorsal root fibres. In the zona marginalis, sensory fibres were also observed, though in an extremely small number. Especially, in sagittal sections stout sensory fibres were found ascending this zone, but it was very doubtful if these fibres further penetrate zona spongiosa, as Kuru has presumed, for I could not observe any sensory fibre running from the marginal into the spongy zone, in my cross sections. The sensory fibres to the nucleus dorsalis seem to include a very few number of sensory fibres forming the ventral part of fasciculus dorsalis, beside the dorsal root fibres penetrating into cornu dorsale.

In conclusion, I will report on my observation of numerous stout fibres running longitudinally through the head of cornu dorsale, close to the ventral side of substantia gelatinosa—an observation obtained in my sagittal sections. Similar fibres seem to have been observed by Lissauer[24] and Kuru,[21] the latter opining that these are formed from the collaterals of the dorsal root fibres, but in my observations, they are represented by the group of ascending sensory fibres formed by the dorsal root fibres themselves.

Summary

Motor cells exist in the lumbar cord not only in cornu ventrale, but also in pars intermedia and cornu dorsale, and they are clearly distinguishable from the vegetative and sensory cells, through that they are very large in size, show a concave contour and are provided with many stout processes, giving them an appearance of rugged virility. Motor cells in

cornu ventrale are especially typical in their form. They have usually one long process each, which is thick and strongly stainable, but seeing that the total number of long processes is larger than that of motor cells on the same cross section, it may be surmised that some cells send out two long processes each, or some of the processes branch out in 2 rami. The short processes number 5 to 7 per cell, are stout but somewhat weakly staining, and after a rather long course, mostly branch out in numerous rami and apparently end freely. Frequently, true anastomosis of short processes is observed between both motor cells.

Motor cells in cornu ventrale may be divided into the medial and lateral groups. The cells in the former group are smaller both in number and size than the cells in the latter group. The former can be divided again into the ventral and dorsal subgroups. The dorsal subgroup is especially poor in development. The lateral group, on the other hand, is very well developed, and can be divided into the ventral, middle and dorsal subgroups, and especially in the lumbar swelling the number of motor cells increases, so that they may be justly subdivided further.

Motor cells in pars intermedia are arranged sporadically, with prevalence in its medial parts, where 3 cells are usually seen on one cross section. In shape, they resemble the small motor cells in the medial group in cornu ventrale. Motor cells in cornu dorsale are seen in its lateral, medial and basal parts, where 1 or 2 each are found in the respective areas. They are also small in size.

Motor cells in cornu ventrale, together with their long and short processes, are found buried in a plexus of incoming nerve fibres. Of the plexus fibres, the motor fibres penetrate into the motor cell group chiefly through the tractus corticospinalis lateralis, the reflex sensory fibres through the entrance zone of dorsal root fibres, and the minute vegetative fibres from the circumference of the cell group. These fibres are all thinner than the short processes of motor cells. The incoming motor and sensory fibres are both better stainable and stouter than the vegetative fibres, so that the distinction between them is readily made. The end formation of these incoming fibres toward the motor cells is neither represented by pericellular reticular formation nor by annular or pin-head shaped corpuscles, as has been held hitherto, but by an intricate criss-cross run in every direction of the incoming fibres. They seem to end in simple branched endings around the short processes, rather than around the cell bodies.

The transmission of stimuli seems to be accomplished by the following process. First, the stimuli carried by the incoming fibres excite the glial cells apparently of endocrinous nature, whose secretion secondarily excites the motor cells, in particular their short processes. The excitement of

the motor cells is then transmitted by the way of the long processes to the periphery.

Sensory cells in the lumbar cord stand midway between the motor and vegetative cells in size, and are morphologically readily distinguishable from the latter two. Only few of them are rounded and feminine in appearance as those in the sensory nuclei in the brain stem, the majority being either spindleshaped or triangular in form. Their short processes are strongly stainable and stout, but stand behind those of motor cells both in number and length. They apparently end without branching or in a few branches, always sharply.

These sensory cells are different in size and shape according to their localisation. For example, in zona spongiosa they are elongated spindle-shaped and arranged in 1 or 2 rows around the substantia gelatinosa. Their short processes are extremely small in number and short in length. The long processes run along the medial and lateral edges of the substantia gelatinosa.

There exist sensory cells in both the dorsal and ventral side of the gelatinous substance. Those in the dorsal side are very few, are shaped like fat pyramids or stars, with short processes ending sharply. The long procesces are deemed to be ascending in course. Sensory cells on the ventral side are rather more numerous, are of slender spindle shape, of which the points are oriented ventrally and dorsally each. The long processes are also ascending and are often bifurcated.

The sensory cells in cornu dorsale are variegated in form—broad spindle-shaped, elongated spindle-shaped, triangular, round—of which the broad spindle cells are comparatively numerous in its medial side, with several short processes, the elongated and triangular cells are found in its lateral side, are smaller than the preceding type both in size and number, with short processes numbering 3 or 4, while the round cells are found in its head and in a much smaller number and similar in form to the sensory cells in the sensory nuclei in tle brain stem.

Sensory nucleus corresponding to the Clarke's nucleus is found also in the upper lumbar cord. But it consists of very few cells, numbering only 3 to 7. The cells are next to the motor cells in size, are provided with 2 to 5 stout short processes and show similarity to the cells in the sensory nuclei in the brain stem. The short processes are branched, ending sharply. The long processes run to the tractus spinocerebellaris dorsalis of the same side.

Nucleus intermediodorsolateralis (Kuru) found in the sacral cord is doubted of its existence in the lumbar cord.

The sensory cells, together with their short processes, lie buried in a complicated plexus. This plexus is composed of incoming sensory fibres

and vegetative fibres. The latter consist of weakly staining minute fibres, while the former are of stout fibres probably ending in simple branched terminations. The transmission of stimuli is effected much in the same manner as by motor cells, primarily from the sensory fibres to the glial cells, and thence, by means of their endocrinous secretion secondarily to the sensory cells.

Of the incoming sensory fibres, those entering cornu dorsale through the entrance zone of dorsal root fibres can be divided into those reaching indirectly after penetrating the substantia gelatinosa and those coming directly from the medial side of cornu dorsale. Into zona spongiosa only a few sensory asending sensory fibres. The fibres going into nucleus dorsalis are originated in the dorsal root fibres entering cornu dorsale and the ventral part of fasciculus dorsalis. In the head of cornu dorsle some longitudinally running sensory fibres originating in dorsal root fibres are found.

References

1) Golgi, Neurol. Zbl., 1890, **5**, 372.
2) Cajal, Arch. Anat. u. Physiol., 1893, 319.
3) van Gehuchten, Les Centre Nerveus, Louvain, A. Uystruyst-Dieudonné, 1908.
4) Lenhossék, Der feinere Bau d. Nervensystems, Berlin, Kornfeld, 19855.
5) Lavdowsky, Arch. mikr. Anat., 1891, **38**, 264.
6) Obersteiner, Anleitung beim Studium d. Baues d. nervösen Zentralorgane, Leipzig u. Wien, F. Deuticke, 1912.
7) Kölliker, Handbuch der Gewebelehre des Menschen, Leipzig, W. Engelmann, 1896.
8) Ziehen, Handbuch der Anatomie des Menschen, Jena, Bardeleben, 1899.
9) Bielschowsky, Neur. Zbl.,.1903, **22**, 997.
10) Edinger, Einführung in d. Lehre v. Bau u. d. Verrichtungen d. Nervensystems. Leipzig, Goldstein u. Wallenberg, 1912.
11) Sato, Tohoku Igaku Zassi (Jap.), 1949, **39**, 151.
12) Atsuki, Tohoku J. Exp. Med., 1953, **58**, 283 & 295.
13) Waldeyer, Das Gorilla-Rückenmark, Berlin, Abh. d. königl. Akad. d. Wiss., 1889.
14) Jacobsohn, Neur. Zbl., 1908, **27**, 617.
15) Stöhr, Lehrbuch der Histologie und der mikr. Anat. d. Menschen, Berlin-Göttingen-Heidelberg, 1951.
16) Yamamoto, Tohoku Igaku Zassi (Jap.), 1948, **38**, 81.
17) Déjérine, Semiologie des affections du systeme nerveux, Paris, Masson et C. Editeurs, 1914.
18) Ranson, The Anatomy of the nervous system, Philadelphia & London, 1936.
19) Kohnstamm, Neur. Zbl., 1910, **29**, 663.
20) Massaza, Arch. d'Anat., d.Histol. et d'Embryol., 1922, **1**, 323.
21) Kuru, Igaku Soho (Jap.), 1949, **12**, 1.
22) Bok, Möllendorffs Handbuch der mikr. Anatomie des Menschen, Berlin, J. Springer, 1928, IV/1.
23) Sano, Arb. aus d. neurol. Institt. d. Wiener Univ., 1909, **17**, 1.
24) Lissauer, Arch. Psych., 1886, **17**, 378.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

On Vegetative Nerve Cells in Human Lumbar Cord

By

Mamoru Suzuki
(鈴木 衛)

From the Anatomical Laboratory of Prof. H. Seto,
Tohoku University, Sendai

(Received for publication, May 14, 1953)

In my previous report, I have detailed my observations on the motor and sensory nerve cells in the human lumbar cord. In continuance of the study, I made a minute examination of the vegetative nerve system, in particular of the vegetative cells, in the same region, using the same preparations impregnated by Seto's method, and arrived at the following observations.

The existence of nerve cells in cornu laterale of spinal cord has been reported by Clarke,[1] Waldeyer[2] and others, but Mott[3] was the first to assert their sympathetic nature. He assumed that the sympathetic nerve fibres running to the periphery through the radix ventralis, as affirmed by Gaskel, have their origin in the intermedio-lateral cell group in cornu laterale. As the results of experimental studies by Biedl,[4] Anderson,[5] Hering[6] and Kai,[7] the vegetative nature of these cells received further support. Kölliker[8] asserted that vegetative cells are found also in the medial side of cornu ventrale.

Studies on the vegetative cells in the spinal cord were continued into this century, of which the results given out by Jacobsohn[9] seem to be looked upon as representative, and many investigators, including Lewandowsky[10] and Oppenheimer[11] have expressed concurrence to his opinions. But a few researchers, e.g. Müller,[12] have registered opposition to some propositions of Jacobsohn,[9] especially concerning the distribution of vegetative cells.

According to Jacobsohn,[9] sympathetic nerve cell group is said to exist in the cornu laterale of the thoracal cord, in the part corresponding to cornu laterale in the cranial lumbar cord, and also in the medial side of cornu ventrale from the 4th lumbar segment downwards to the sacral cord, which he called nucleus symp. lat. sup., nucleus symp. lat. inf. and nucleus symp. med. cornu anterioris respectively. Müller observed sympathetic nuclei in cornu laterale of the caudal thoracal cord and the

lateral side of pars intermedia of the cranial lumbar cord, and parasympathetic nucleus in the lateral side of pars intermedia of the segments 2 through 5 of the sacral cord, of which the latter he identified with the nucleus symp. lateralis inferior of Jacobsohn, but he denied the existence of Jacobsohn's sympathetic nucleus in the medial side of cornu ventrale. Kure[13] supposed existence of spinal parasympathetic nuclei ranged from substantia gelatinosa to pars intermedia, as the result of his experiments on dogs. As cited above, opinions are divided on the vegetative nuclei in the spinal cord.

Now, my observations showed that the vegetative cells in the lumbar cord are much smaller in size than the motor cells and sensory cells. Their shape is also much varied, some being triangular, some spindle-shaped and some others club-like etc., but the majority is stellar in form. The neurofibrils in the cell body are less stainable and more minute than those in motor cells and sensory cells. Thus the vegetative cells may be easily distinguished from the motor and sensory cells histologically. On fruther scrutiny, their cell nucleus is found to be rather large in proportion to the protoplasm and located excentrically. It is of interest that the cell nucleus of some vegetative cells is much larger than those of the motor and sensory cells, even showing macromorphism in some cases.

Nerve cells with macro-sized cell nucleus have been recently observed by Atsuki[14] of this laboratory in the Edinger-Westphal nucleus in nucleus n. oculomotorii. He assumed that these cells are of vegetative nature, and inferring from the specific nerve cells provided with macro-sized cell nucleus and the results of past experiments, consigned them to the parasympathetic nerve system. I concur in his opinion and assume the cells with macro-nucleus to belong to the parasysmpathetic and those with small ordinary-sized nucleus to the sympathetic nerve system.

I observed that in cornu laterale of cranial lumbar cord corresponding to the Jacobsohn's nucleus sympathicus lateralis (I acknowledge, as described hereunder, the existence of sympathetic nerve cell groups in the cranial lumbar cord in the affirmative, and consequently the existence of cornu laterale also here as well as in the thoracal cord) there exist rather large specimens of vegetative cells arranged in groups. These cell groups may be divided into the lateral (Fig. 1), the medial (Fig. 2) and the dorsal adjacent to the formatio reticularis. But the arrangement it not always symmetrical and in some cases the division into three groups is not distinct.

These cells send out only 2 to 4 nerve processes in total, the short processes being much shorter than those of the motor and sensory cells, terminating either unbranched or in simple branched endings, and always sharply. The long processes are said to go over into radix ventralis,

Fig. 1

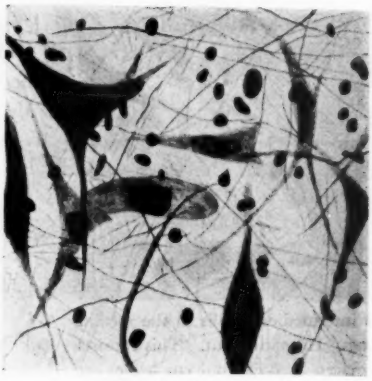


Fig. 2

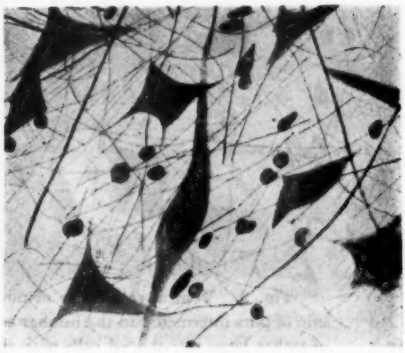


Fig. 1. Sympathetic lateral cell group in cornu laterale of the upper lumbar cord. Human infant. Cross section. Seto's silver impregnation. X 600, reduced to 1/2.

Fig. 2. Sympathetic medial cell group in cornu laterale of the upper lumbar cord. Human infant. Same staining. X 600, reduced to 1/2.

according to Ziehen[15] and Bok,[16] and I regret to say that I could not make out the course of the long processes with definition, but seeing that there are found a rather large number of minute vegetative fibres beside the stout motor fibres in the radix ventralis, it may be true that the long processes of these cells do run to the periphery through the radix ventralis. These cells are all provided with smaller cell nucleus, as seen in the illustrations, and never with macro-nucleus, so that it may be assumed that they are sympathetic in nature.

In the medial side of pars intermedia of lumbar cord are found a vegetative cell group, that may be called nucleus intermediomedialis (Bok).[16] The nerve cells in it have been called Mittelzellen by Waldeyer,[2] and Zwischenzellen by Ziehen.[15] Many researchers have also confirmed their existence. But opinion is not uniform on the function on this nucleus. For example, Ziehen believed these cells to be tract cells, Zdislaw[17] and Marburg[18] assumed their correlation with the tracus cerebellospinalis ventralis and Okinaka[19] experimentally observed retrogression of these cells upon excision of the radix ventralis, and assumed them to be of extrapyramidal nature.

My observation on these cell group has been as follows. These cells are best developed in the lumbar swelling, comprising the ventral groups consisting of cells comparatively large for vegetative cells (Fig. 3), the middle group (Fig. 4) and the dorsal group (Fig. 5) consisting of small vegetative cells. In the cranial and caudal parts, the arrangement

Fig. 3

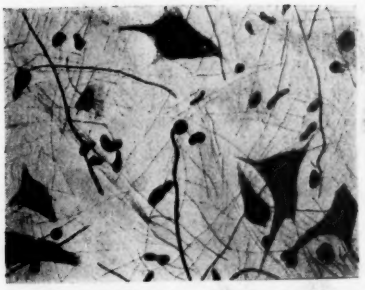


Fig. 4

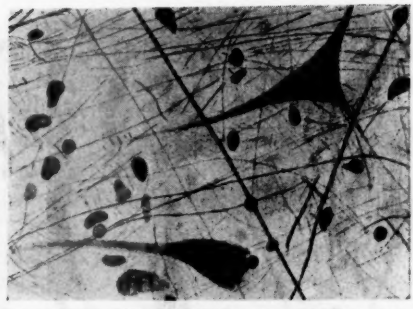


Fig. 3. Ventral cell group of nucleus intermediomedialis in the medial side of pars intermedia in the lumbar swelling. Human infant. This consists of rather large star-shaped cells with their macro-sized cell nucleus belonging to the sympathetic nerve system. Cross section. Same staining. X 600, reduced to 1/2.

Fig. 4. Middle cell group of nucleus intermediomedialis in the lumbar swelling. Human infant. Nerve cells are parasympathetic cells of triangular or spindle form and with their rather large cell nucleus. Cross section. Same staining. X 600, reduced to 1/2.

in three groups becomes indistinct. The large cells in the ventral group are stellar in shape and have the above mentioned macro-sized cell nucleus, presumably parasympathetic in nature. They have weakly developed 3 or 4 short processes each, and long processes, of which the running cours has not yet been adequately pursued.

The small cells in the middle and dorsal groups are chiefly triangular or spindle-shaped, are provided with not macro-sized but yet comparatively large and strongly stainable cell nucleus, so that the cell bodies appear very narrow. These cells also I presume to be parasympathetic. Nerve processes are very limited in number and the running course of the long processes could not be surveyed with clarity.

A band-formed grey substance zone running from the dorso-medial edge of cornu ventrale along the dorso-lateral edge of the fasciculus ventralis toward the commissura ventralis grisea, containing sporadic vegetative cells, has been observed, which seemingly corresponds to the nucleus cornu commissuralis anterioris of Bok[16] and Massaza.[20] The cells here (Fig. 6) are small in size, shaped somewhat elongated-triangular, polygonal or stellar in general, but sometimes also spindle-shaped. The long axis of the cells is approximately parallel to that of the zonal nucleus and their nerve processes generally run parallel thereto, running a rather long course. Some cells apparently belonging to this nucleus are found wandering into the ventral side of canalis centralis. This nucleus is

Fig. 5

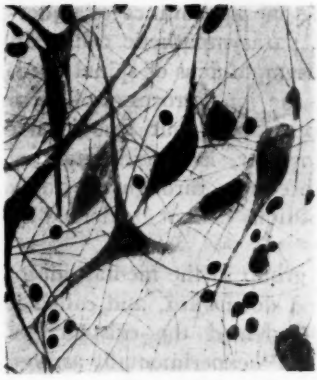


Fig. 6

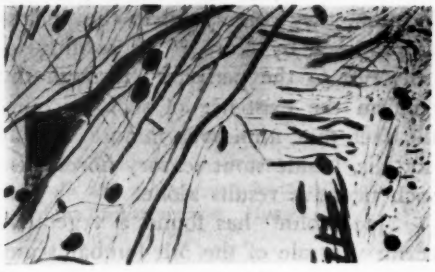


Fig. 5. Dorsal cell group of nucleus intermediomedialis in the lumbar swelling. Human infant. Nerve cells are similarly sympathetic in nature. Cross section. Same staining. X 600, reduced to 1/2.

Fig. 6. Triangular-shaped parasympathetic nerve cells seen in nucleus cornu commissuralis anterioris. Human infant. Cross section. Same staining. X 600, reduced to 1/2.

also deemed to belong to the parasympathetic system, for the cell nucleus is mostly rather large. However, as some cells with smaller cell nucleus are seen included in this nucleus, it is probable that also sympathetic cells are coexistent.

The Bok's so-called nucleus cornu commissuaralis posterioris is seen describing an arc from the approximate center of the medial edge of the head of cornu dorsale to commissura dorsalis grisea, containing generally small cells arranged sporadically, spindle-shaped with their long axis with few exceptions parallel to that of the arc-form nucleus and their bipolar processes also running in general parallel thereto. The nerve cells having comparatively large cells nucleus, this nucleus also apparently belongs to the parasympathetic system.

I do not recollect of having read a detailed description of nerve cell group in the substantia grisea centralis of the dorsal side. In this area I have observed the existence of several vegetative cells, especially rather large cells with comparatively strongly developed processes, stellar or polygonal in shape, through the cross sections. As these cells are provided with large and sometimes oversized nucleus, I presume this nucleus also belong to the parasympathetic system and propose the name of nucleus dorsalis canalis centralis.

In the substantia gelatinosa and cornu dorsale are found scattered minute vegetative cells, in most cases polygonal, especially triangular,

in shape. These cells have the peculiarity of having a very large cell nucleus in proportion to the cell body, giving the appearance of undersized cell body. In form, they are typical parasympathetic cells. In location, they correspond to the nucleus parasympathicus of spinal cord, as proposed by Kure.[13] According to him, the long processes of these cells run to the periphery through the radix dorsalis, but I could not confirm the existence of such a course. However, in consideration of the fact that numerous minute vegetative fibres run through the radix dorsalis, beside stout sensory fibres, his propositions cannot be denied by such negative results alone.

Jacobsohn[9] has found a vegetative cell group in the medial side of cornu ventrale of the 5th lumbar segment and downward, and called it nucleus sympathicus mediosacralis. Müller[12] denied the existence of such a group, but Kölliker[3] affirmed it and Nitta[21] experimentally arrived at the conclusion that it apparently represents a genital nerve centre.

I could not only confirm the existence of this vegetative nucleus, but also found that these vegetative cells extend much more cranial into the top part of the lumbar swelling. The nerve cells in it are polygonal or triangular in shape and varied in size, as shown in Fig. 7, and can be classified into those having macro-sized cell nucleus and those with comparatively smaller cell nucleus. This nucleus is thus assumed to be both sympathetic and parasympathetic in nature.

Bok[16] says in his so-called nucleus proprius cornu anterioris is to be found between the motor nucleus medialis and nucleus lateralis in cornu ventrale of lumbar cord. According to my investigation, there exist also vegetative nerve cells in the motor cell groups. They do not show, however, such a localized existence of vegetative cells, as Bok has described, but are almost evenly scattered throughout the entire area of all the motor cell groups. They are never abundant in number, varied in size, and have either large or small cell nucleus, so that they are also parasympathetic and sympathetic in nature, like the nucleus sympathicus mediosacralis.

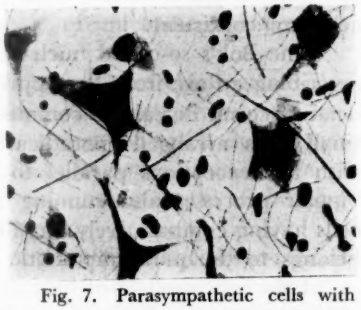


Fig. 7. Parasympathetic cells with large cell nucleus and sympathetic cells with small cell nucleus in Jacobsohn's so-called nucleus sympathicus mediosacralis in the lower lumbar cord. Human infant. Cross section. Same staining. X 600, reduced to 1/2.

Lastly, some sporadic existence of vegetative cells in the formatio reticularis is observed. The function of these cells has been unclarified,

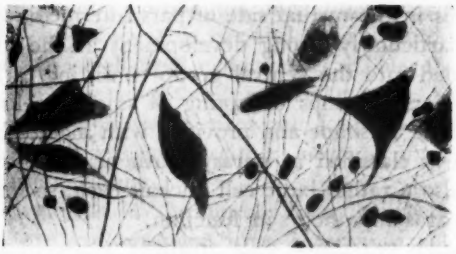


Fig. 8. Many parasympathetic cells and a few sympathetic cells in formatio reticularis in the lower part of the lumbar swelling. Human infant. Cross section. Same staining. X 600, reduced to 1/2.

but seeing that the majority of the cells is triangular or spindle-like in shape, and has a large or macro-sized cell nucleus each, it is assumed that they belong to the parasympathetic system (Fig. 8).

In concluding my detailed description of the vegetative nerve cells found in the lumbar cord, I may point out the interesting fact that these cells do not form one continuous column of nucleus through the lumbar segments but are arranged in disjointed groups. This fact has been particularly apparent in my sagittal sections.

Summary

Vegetative cells in lumbar cord are much smaller than motor and sensory cells in it, poorer in nerve processes and stellar, triangular, spindle or club-shaped in form. Nerve fibrils in the cell body are also much less stainable and minuter than those in motor and sensory cells. The cell nucleus is, however, larger than in the motor and sensory cells, sometimes macro-sized.

These vegetative cells with oversized cell nucleus were proved by Atsuki also in the Edinger-Westphal's nucleus, and are assumed to be parasympathetic in collation with experimental results. In accordance with him, I also assume that the cells with large cell nucleus are parasympathetic and the cells with smaller one are sympathetic in nature.

In cornu laterale of the cranial lumbar cord, some larger vegetative cells are found, divided into lateral, medial and dorsal groups. The short processes of these cells are very short and small in number, ending sharply in most cases. I could not follow the course of the long processes to an adequate distance, but they may go over into radix ventralis, as assumed by Ziehen and Bok, for many minute vegetative fibres are found incorporated in radix ventralis. These cells are deemed sympathetic, as having a small cell nucleus each.

There exist some vegetative cells corresponding to Bok's nucleus

intermediomedialis in the medial side of pars intermedia of lumbar cord. They come to particularly strong development at the lumbar swelling, and can be divided into the ventral group consisting of larger cells and the medial and dorsal groups consisting of smaller cells. The cells in the former are stellar in shape and provided with macro-sized cell nucleus and can be clearly classified as parasympathetic cells, but those in the latter two have also a rather large cell nucleus each, so that they are also assumed to be parasympathetic in function.

There are some vegetative cells arranged scattered in a band from the dorsomedial side of cornu ventrale to the commissura ventralis grisea, which seemingly correspond to the nucleus cornu commissuralis anterioris (Bok). The cells are small, with shape of elongated triangle or polygon, star or spindle, with their long axis and nerve processes parallel to that of the nucleus. These cells are provided with large cell nucleus and are apparently parasympathetic. But sometimes sympathetic cells with smaller cell nucleus are also found coexistent with the parasympathetic.

The Bok's nucleus cornu commissuralis posterioris is found diffused from the medial side of the head of cornu dorsale to the commissura dorsalis grisea, consisting of small-sized spindle-shaped parasympathetic cells with rather large cell nucleus. Their long axis and their generally bipolar processes are arranged parallel with the arcuate long axis of the nucleus. In the dorsal side of substantia grisea centralis are also found rather large stellar or polygonal cells grouped in several units. They are parasympathetic cells with large or macro-sized cell nucleus, and I propose to call their group nucleus dorsalis centralis.

In the substantia gelatinosa and cornu dorsale are found scattered polygonal minute vegetative cells. Their cell nucleus is very large in proportion to the cell body. This cell group is thus presumed to be parasympathetic and seems to correspond to the nucleus parasympathicus of spinal cord as shown in the experimental study of Kure. He says that the long processes of these cells run to the periphery through the radix dorsalis, but I could not follow up their course satisfactorily. However, as many minute vegetative fibres are found running through the radix, I am not in the position to deny his assertion.

Nucleus sympathicus mediosacralis, observed by Jacobsohn in the medial side of cornu ventrale of the 5th lumbar segment and downwards, is found in my specimens diffused as far as into the lumbar swelling. The nucleus consists of polygonal cells of variable size, and is divisible into cells with macro-sized and those with smaller cell nucleus. The group seems to enjoy both of the sympathetic and parasympathetic functions.

Vegetative cells are also found in the motor cell groups in cornu ventrale, but these are not localized in existence, as Bok would hat it,

but are dispersed evently throughout the entire area of all the motor cell groups. They are of variable size and divisible into the two cell types with macrosized and small-sized cell nucleus. Thus their dual function of sympathetic and parasympathetic natures may be inferred.

Vegetative cells of variable sizes are found scattered in formatio reticularis. Their cell nucleus is either large or macro-sized, showing their parasympathetic nature.

The vegetative cells in the lumbar cord described above, as seen in sagittal sections, are not arranged in a continuous column, but divide into disjointed groups.

References

1) Clarke, Philos. Transact. Roy. Soc. Lond., 1859.
2) Waldeyer, Das Gorilla-Rückenmark, Abh. d. königl. Academie d. Wiss. Berl., 1889.
3) Mott, Brain, 1890, **13**, 433.
4) Biedl, Wiener Klin. Wschr., 1895, **52**, 951.
5) Anderson, J. Physiol., 1894, **16**, 12.
6) Hering, ibid., 1903, **29**, 282.
7) Kai, Tokyo Igakkai Zassi (Jap.), 1923, **38**, 1423.
8) Kölliker, Handb. d. Gewebelehre d. Menschen, Leipzig, W. Engelmann, 1886.
9) Jacobsohn, Neurol. Zbl., 1908, **27**, 617.
10) Lewandowsky, Handb. d. Neurologie, Berlin, J. Springer, 1910.
11) Oppenheimer, Lehrbuch der Nervenkrankh., Berlin, S. Karger, 1913.
12) Müller, Die Lebensnerven, Berlin, J. Springer, 1924.
13) Kure, Ueber den Spinalparasympathicus, Basel, B. Schwabe, 1931.
14) Atsuki, Tohoku J. Exp. Med., 1953, **58**, 283 & 295.
15) Ziehen, Handb. d. Anatomie d. Menschen, Jena, G. Fischer, 1899.
16) Bok, Möllendorff's Handb. d. mikr. Anat. d. Menschen, IV/1, Berlin, J. Springer, 1928.
17) Zdislaw, Arb. a. d. Wiener neur. Inst., 1909, **17**, 314.
18) Marburg, Mikr.-topogr. Atlas d. menschl. Zentralnervensystems, Leipzig u. Wien, F. Deuticke, 1927.
19) Okinaka, Tokyo Igakkai Zassi (Jap.), 1932, **46**, 1006.
20) Massaza, Arch. d'Anat., d'Histol. et d'Embryol., 1922, **1**, 233.
21) Nitta, Tokyo Igakkai Zassi (Jap.), 1929, **43**, 610.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

Chemistry and Biology of Lipids

XIV. Group Lipoid of Pig Stomach Mucosa

By

Masahiro Tokura
(登倉 正博)

From the Medico-chemical Institute, Tohoku University, Sendai;
Director: Prof. H. Masamune

(Received for publication, May 14, 1953)

The group lipoid in question was separated in an electrophoretically homogeneous state. A lipid mixture of mucosa of pig stomachs was fractioned first by the aid of organic solvents. And, next, the crude product was subjected, as taken up in benzene, to fractional adsorption on Al_2O_3 column. The part remaining unadsorbed was distinctly Group A active. Since, however, electrophoresis in an alkaline buffer, showed it inhomogeneous, demonstrating two boundaries far remote from each other, division into the components was effected in the same apparatus. The major component was the one desired. It dissolved well in hot benzene, in hot tetraline and in hot alkaline water (pH 9.0). Analyses: N 2.1%, P 2.2%; total reduction calculated as glucose 17.4%, ash 10.4%. It possessed Group O activity besides, though weak.

Experimental

Preparation Procedure

Fractionation of a lipid mixture of pig stomach mucosa (proper) was carried out, taking the group activity of the divisions as an indication.

1st stage: Fresh mucosa (freed from mucus) pulp of 24 pig stomachs (4185 g. altogether) was stood for 7 days as covered under 4.5 volumes of 91% denatured alcohol containing 3% methanol, squeezed, and dried up in a Faust apparatus—788 g. The material thus obtained was boiled twice with 5 volumes of 96% denatured alcohol for 2 hours and the solutions were percolated, while hot, and placed in an ice-chest overnight. The occurring precipitates were discarded and the mother fluids were distilled together in vacuo to dryness and washed with acetone. 4.2 g. of a yellow, hygroscopic powder were given, which showed Group A activity at a dilution of $1:10^5$.

The substance was next washed many times with ether and dried. 2.2 g. of a yellowish hygroscopic powder remained. The group activity

was found scarcely raised. (The washings were vacuum-distilled. The still residue proved almost inactive, inhibiting the isoagglutination of A erythrocytes only at a dilution of $1:5\times10^2$.) It was then treated with three 40 cc. portions of cold abs. alcohol. And the insoluble (1.7 g.; Group A active dilution, $1:10^5$) was further exhausted by boiling with benzene for 5 minutes under reflux, followed by centrifuging while hot. The solution was distilled and the dried up residue was washed with acetone. 1.3 g. of a somewhat yellow, non-sticky powder were given—Prep. I. The Group A active dilution was again found to be $1:10^5$.

2nd stage. Commercial alumina (Al_2O_3) was washed under running water and ignited in a nickel dish for 2 hours. Three columns of 1.2 cm. inner diameter were packed with the activated aluminium oxide as high as 20 cm. On the other hand, 1.05 g. of the product above were heated with benzene, and when cold, 70 cc. portions of the solutions (in total 210 cc.) were separately run through the columns. After washing down all the fluids with an equal volume of benzene, the effluents were distilled together in vacuo to dryness, collected with acetone and dried. A yellowish non-stricky powder given weighed 170 mg.—Prep. II. This showed inhibition to isoagglutination of A erythrocytes at a dilution of $1:4\times10^5$. Examination at pH 9.0 (a carbonate buffer) in a Tiselius apparatus demonstrated it to comprise two components, as is seen in Fig. 1.

The column above was followingly washed with pyridine, methanol, ammoniacal methanol (pH 9.0) and water in succession, but in the washings no appreciable amount of the anti-agglutinative factor was present. Probably the factor in benzene is scarcely adsorbed on Al_2O_3.

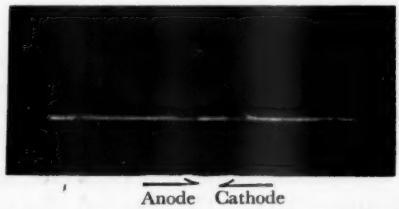


Fig. 1. An electrophoretic pattern of a 0.86% solution of Prep. II in carbonate buffer, pH 9.0, I=0.105 (The substance was dissolved in the buffer by short heating at 40°C. The solution given was opalescent); 13°C; 10 ma; exposure 45 minutes; mobility of the first moving boundary 13.8 $cm^2 \cdot volt^{-1} \cdot sec^{-1} \cdot 10^5$. Ascending limb to the left.

3rd stage. 113 mg. of Prep. II were dissolved in 12 cc. of the carbonate buffer above and fractioned into the components electrophoretically in a three-segmented cell. The portion of the solution containing the

faster moving component, the major component, was sucked out and dialyzed against changes of distilled water, then it was condensed by vacuum distillation and the residue was collected with acetone and dried. 32 mg. of a light yellow powder were given—Prep. III. Electrophoresis of it showed a single boundary corresponding to the faster moving in Prep. II. The slower moving was recovered from the solution portion remaining in the cell in a similar manner. 10 mg. of a yellowish powder were given which was Group A active only at 1:500 dilution and Group B inactive even at a dilution of 1:200.

Remarks. Preparatory experiments for devising the 2nd stage procedure were made on a batch of substance like Prep. I. Some of the trials that were useless, will be appended below, because it may be serviceable for investigators. 1) A solution of the substance in benzene was passed through a MgO[1] column and then the substance adsorbed treated with methanol, but most of the still residue of the eluent did not dissolve in a phosphate buffer of pH 7.8, even when heated to boiling. 2) Next the methanol above was substituted with pyridine for the elution of the active lipid but in vain. After pyridine, methanol was poured on the column, but the fraction recovered from the present effluent again proved insoluble in the phosphate solution. 3) The substance was subjected to counter current distribution with a solvent pair consisting of an equilibrated mixture of water, methanol, benzene and toluene (1:4:4:1 by volume) as in the former investigations regarding the group lipoids of pancreas,[2] but the highly active portions put together (Group A active at a dilution of $1:5\times10^4$) showed three closely-neighbouring boundaries on electrophoresis at pH 9.6.

Properties of the Final Product

Group activities. It was Group A active at a dilution of $1:10^5$, and Group O active at a dilution of $1:5\times10^3$, but Group B inactive (examined at a dilution of 1:500). All the potencies were measured by determining the maximum dilution where the substance was inhibitory to agglutination of human erythrocytes. Details are seen in Tables I and II. Thus it appeared to be a mixture of Group A and Group O substance, consisting mainly of the former.

Analyses. N (micro Kjeldahl), 2.1%; P (a modification of Plimmer method[3]), 2.2%; Equivalent ratio of N to P $\fallingdotseq$ 2.1:1.0. Reduction of ferricyanide after hydrolysis (1 *N* H_2SO_4, 100°C, 4 hrs.), 17.4% as glucose; ash (Pregl method without use of H_2SO_4), 10.4%.

Tests. Biuret negative; Molisch positive; Hexosamine assay after the determination method of Blix[4] positive.

Solubilities. As is shown in Table III, it was completely soluble in

Table I

Occurrence of Isoagglutination of Erythrocytes by the Sera treated with the Group Lipoid Preparation (Prep. III)

The B and A serum had agglutinin titre 320 and 720 and were diluted 20 and 45 times respectively. −: no agglutination, ±: faint aggl., +: slight aggl., ++: marked aggl., ###: strong aggl.

Group of red cells	Dilution of Prep. III (1:)										
	5×10^{2}	10^{3}	5×10^{3}	10^{4}	5×10^{4}	10^{5}	2×10^{5}	4×10^{5}	8×10^{5}	1.6×10^{6}	control*
A	−	−	−	−	−	−	±	+	+	+	++
B	+	+	++	###	###						###

* The sera were not treated with the substance in advance.

Table II

Occurrence of Agglutination of O Erythrocytes by an Eel Serum treated with Prep. III

The serum had agglutinin titre 8×10^{2} towards O erythrocytes but did not agglutinate A and B erythrocytes at a dilution of 1:25, and was diluted 50-fold with physiological saline for the assay. −: no agglutination, +: slight aggl.

Group of red cells	Dilution of Prep. III (1:)										
	2×10^{2}	5×10^{2}	10^{3}	5×10^{3}	10^{4}	5×10^{4}	10^{5}	2×10^{5}	4×10^{5}	8×10^{5}	control*
O	−	−	−	−	+	+	+	+	+	+	+

* The serum was not treated with the substance in advance.

Table III

Solubilities of Prep. III

−: insoluble, ±: faintly sol., +: partly sol., ++: mostly sol., ###: completely sol. cold=5°C, hot=heated to boiling excepting the case of tetralin where the mixture was warmed to 60°C.

Solvent	Plain water		Acidic water†		Alkaline water‡		Glacial acetic acid		Methanol		Ethanol		Ether		Petrol ether		Acetone		Chloroform		Tetralin		Benzene	
	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot
Solubility	++*		±	+	+	##*	−	±	±	+	±	+	−	−	−	−	−	−	±	++	±	##	+	##

* An opalescent solution was given. † A dilute HCl solution of a pH of about 1.0. ‡ A dilute NaOH solution of a pH of about 9.0.

hot benzene and tetralin. It dissolved entirely in water alkalinized to pH 9.0 with NaOH when heated to boiling, giving an opalescent solution.

Summary

From a batch of stomach mucosa of pigs was obtained a lipid preparation which was demonstrated to be essentially hemogeneous after electrophoresis in an alkaline buffer. It was quite strong in Group A activity but possessed also Group O activity.

Through the Grant Committee for Scientific Researches was given a grant from the Education Department in aid to us. H. Masamune.

References and Notes

1) Prepared by heating freshly precipitated $MgCO_3$ at $>420°C$ for 3 hrs.

2) Tokura, Tohoku J. Exp. Med., 1952, **56**, 299 and 307; Masamune, Tokura & Satoh, *ibid.*, 1952, **57**, 21.

3) Haneda, Sinokawa & Yasuoka, *ibid.*, 1944, **46**, 263.

4) Blix, Acta chem. Scand., 1948, **2**, 467.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

Biochemical Studies on Carbohydrates

CLXII. High-molecular Components of Pig Kidney-cortex, particularly a Carbohydrate or Carbohydrates Containing Hexosamine

By

Genji Kikuchi
(菊池 源二)

From the Medico-chemical Institute, Tohoku University, Sendai;
Director: Prof. H. Masamune

(Received for publication, May 14, 1953)

Pig kidney-cortices were extracted with a saline solution after dehydration with alcohol in one case and in the other immediately. And, by fractionation of the solutions two biuret negative carbohydrate-preparations were obtained which proved inhomogeneous after electrophoresis, but analyzed similar to each other. In either of them, the nitrogen content amounted more than twice the calculated from the hexosamine content, suggesting a complex or complexes of a polypeptide and a polysaccharide. Whether the preparations were mixtures of differently aggregate molecule of one carbohydrate or those of different carbohydrates is not decided for the present. The details regarding the preparation procedure and the properties of the products will be described below.

Experimental

Preparation Procedure

1. 50 pig kidneys were taken. The cortices (4.8 kg.) separated mechanically were ground in a masticator, stood as covered under 4.5 volumes of denat. alcohol containing 3.5% methanol for 5 days and then dried in a Faust apparatus. 125 kg. of the material thus obtained were boiled with 5 and 3 volumes of abs. alcohol in sequence for 2 hours each. The residue was dried as above. 0.97 kg. was the amount. It was divided into three equal portions for successive treatments as below: The divisions were mixed with 10 volumes of a 10% saline solution and the mixtures shaken for 3 days (Toluene was used as a preservative). The extraction was repeated three times, followed by centrifugation until none came out anymore. The supernatant fluids were treated separately again. The description below concerns the first centrifuged supernatant in the case of the first division of the defatted material. It (a somewhat turbid, light brown supernatant, 2.7 *l.*) was acidified with 10% HCl to

a pH of 1.5 and centrifuged.

The voluminous centrifugate was washed with a 10% NaCl solution, which had been acidified to pH 1.5 with HCl, and, then, after vigorous agitation in 10% NaCl, neutralized with NaOH (10%). The insoluble part was rejected and the solution was precipitated with 5 volumes of abs. alcohol. The deposit was washed with abs. alcohol and dried—**Fr. I** (1.3 g.). Molisch +; biuret ++; Neuberg-Saneyoshi (for hexuronic acid) +(weak); Goldschmiedt (for hexuronic acid) ±. P ++; purine +; Bial-Mejbaum (for pentose) +; Dische (for desoxypentose) +(weak). Thus this Fr. proved to be a mixture of proteins, nucleic acids (mostly a pentosenucleic acid) and a small amount of a hexuronic acid-containing polysaccharide (polyuronic acid). From its watery solution, freed from the insoluble part, the nucleic acids were carried down with barium acetate and baryta, and the centrifuged supernatant was removed from barium with H_2SO_4, dialyzed and condensed, followed by addition of alcohol. The deposit here showed only weak Molisch reaction contrary to strong biuret reaction. It was further divided into the precipitable and unprecipitable by adjusting its solution to pH 4.2 with HCl. But these subdivisions were not more than faintly Molisch-positive. Thus glucidamins appeared absent among the proteins in Fr. I.

The clear, light brown supernatant was dialyzed for 3 days and some precipitate occurring thereby was discarded. The clear mother fluid was distilled in vacuo to about 30 cc., freed from some precipitate that had come out during the condensation, and 2.5 cc. of 20% barium acetate were added in small portions, until no more precipitation. The voluminous flocculent deposit was centrifuged off.

The centrifugate was washed with 1.6% barium acetate and suspended in a little water and after changing into sodium salts by agitation with portions of a sodium sulfate solution, filtered. And the filtrate was dialyzed and condensed and alcohol added. The precipitate, washed with alcohol and dried, (a white powder) weighed 0.7 g.—**Fr. II.** Molisch +; biuret + (weak); Neuberg-Saneyoshi, Goldschmiedt and Dische −; P ++; purine +; Bial-Mejbaum ++. Hence it consisted chiefly of a pentosenucleic acid

To the supernatant was added the saturated $Ba(OH)_2$ to pH 8.0.

An occurring white flocculent precipitate was also transformed into sodium salts similar to above. 0.75 g. of a white powder was given—**Fr. III.** Molisch and biuret +(weak); P ++; purine ++; Bial-Mejbaum ++; Dische +. From these consequences of tests, the fraction is regarded to have contained pentose- and desoxypentose-nucleic acid as the main components.

The mother fluid was neutralized with 2% HCl and dialyzed for 4 days until no more barium ions were detected. After centrifuging off of some deposit that had occurred during the dialysis, the solution was distilled in vacuo to a syrup, precipitated with 5 volumes of abs. alcohol

and washed with the alcohol repeatedly, adding sodium acetate when requisite. 1.2 g. of a slightly brown powder were given.—**Fr. IV** (**Cr. Ch.**).

Frs. IV's from the whole defatted cortex material amounted to 4.8 g. They were combined and assayed. Molisch ##; biuret ##; Bial-Mejbaum +; Neuberg-Saneyoshi, Goldschmiedt, Dische and tests for P and purine —. The balance of the batch was taken up in 75 cc. of water (pH of the solution, 5.6), saturated with ammoniumsulfate (a voluminous precipitate occurred) and after standing overnight, filled up to 200 cc. with the saturated $(NH_4)_2SO_4$. Centrifuged. The supernatant fluid was freed from suspended particles by filtration.

The centrifugate was dissolved in a small quantity of water, and re-precipitated by saturation with $(NH_4)_2SO_4$. After further addition of the saturated $(NH_4)_2SO_4$ up to 100 cc., the mixture was centrifuged. And the centrifugate was dissolved in 600 cc. of water (A brown, slightly turbid solution was given, whose pH was 6.0) and dialyzed for 5 days when NH_4- and SO_4-ions were not detected. A small precipitate during the dialysis was rejected, and the solution distilled in vacuo to about 40 cc., followed by addition of 10 volumes of alcohol and a little anhydrous sodium acetate. The deposit was washed with alcohol and dried. A light brown powder given was 3.1 g.—**Fr. V.** Molisch +(weak); Bial-Mejbaum ±; biuret ##; Neuberg-Saneyoshi, Goldschmiedt, Dische and test for P —. The present fraction thus looked to be a mixture of water-soluble, carbohydrate-poor proteins. It was further treated by the procedures of Kerr and Seraidarian[1] and Sevag. But the divisions also showed only weak Molisch reaction, which suggested no glucidamins.

The filtrate was diluted fourfold with water and dialyzed against running water for 5 days. Since it proved to still contain SO_4-ions, it was condensed to about half the volume by vacuum distillation and dialyzed again. The sulfate-free solution was concentrated as above to about 30 cc. Then the slightly turbid, light yellow solution of pH 6.0 was subjected to centrifugation and the clear supernatant was precipitated with abs. alcohol containing some sodium acetate, washed with alcohol and dried in vacuo over $CaCl_2$. A white powder given weighed 0.83 g. Molisch ##; biuret #, Bial-Mejbaum +.

0.82 g. of the product was then dissolved in 20 cc. of water and 10% lead acetate was added carefully to just carry dwon all the precipitable (2.5 cc. of the salt solution were required). Stood for 5 hours and centrifuged.

The precipitate liberated from lead by the aid of H_2SO_4 and H_2S—**Fr. VI**—showed only slightly positive Molisch but strong biuret reaction. The presence of glucidamins was again disproved.

The supernatant, which was somewhat turbid, became clear on re-centrifugation after standing overnight in an ice chest. It was then bubbled with H_2S and the black sediment filtered off. The filtrate was vacuum-distilled to a syrupy solution, precipitated with 5 volumes of abs. alcohol and after washing with the alcohol, dried. 0.36 g. was the white powder yielded. Molisch ##; biuret ±; Bial-Mejbaum +.

Finally the balance (0.35 g.) of the product was kneaded with 1 cc. of water and to the viscid solution were added 11.25 cc. of glacial acetic acid (b.p. 117–118°C, 98%). The flocculent sticky precipitate was centrifuged and washed with 90% acetic acid twice and further with ether five times and dried—**The Acetic Acid-Insoluble I (Ch. I)**. Crop, 180 mg. To the light yellow supernatant were added three volumes of ether and the centrifugate also washed with ether thoroughly and dried—**The Acetic Acid-Soluble I (Ch. I')**. Crop, 140 mg. Either of these fractions was a white powder. The Acetic Acid-Insoluble I: Molisch ##, Bial-Mejbaum +, biuret, Neuberg-Saneyoshi, Goldschmiedt, Dische, Osaki-Turumi (the direct as well as the indirect), ninhydrin and Masamune *et al.* $HgCl_2$ test[1a] —, hydrolysable sulfur, phosphorus and purine —. The Acetic Acid-Soluble I did not differ in test-tube tests from above, but that it gave weak biuret reaction.

2. In this case a fresh tissue material was immediately treated with physiological saline and the solutions fractioned as above with a few modifications.

The cortex of 5 pig kidneys, weighing 0.6 kg. in total, were masticated and shaken with 3 *l.* of a 0.85% saline solution for 12 hours, using toluene as a preservative. Centrifuged. (The centrifugate was subjected to further extractions.) To the supernatant fluid, which was turbid and red-brown colored, was added 10% HCl in small portions to pH 4.5. (The voluminous flocculent precipitate was separated off for preparing the pentosenucleic acid which will be reported elsewhere.[2]). The nearly clear mother fluid was dialyzed for 2 days. A pretty large amount of a precipitate came out thereby. This was rejected. The fluid was distilled in vacuo to about 200 cc., whereby again precipitation occurred. After removing of the deposit, it was further concentrated to syrupy consistency, precipitated with 5 volumes of abs. alcohol and washed with the alcohol, followed by drying in vacuo over $CaCl_2$. A brown powder given weighed 5.0 g. and gave the strong biuret reaction. It was also Molisch positive but Neuberg-Saneyoshi and Goldschmiedt negative. Phosphorus was detected. After the second extraction of the same material, 0.5 g. of a similar substance was given. 5 more runs were carried out, employing 2.89 kg. of fresh cortices in all, and 35.1 g. in total of the products were obtained. They were combined together—**Fr. A.**

5.5 g. of Fr. A were taken up in 50 cc. of water. The insoluble part was filtered off. To the filtrate were added 4.2 cc. of 20%barium acetate until no more precipitation occurred, then to the centrifuged supernatant was added the saturated baryta to pH 8.0. The centrifuged supernatant was neutralized with HCl, dialyzed for 4 days against running water to eliminate SO_4-ions and after rejecting some sediment, distilled in vacuo. The remaining thick solution was precipitated and washed with abs. alcohol and dried. A brownish powder given amounted to 2.1 g. It was perfectly free from phosphorus. Molisch and biuret positive. Dische negative. From the whole Fr. A were yielded 11.1 g. of the present fraction—**Fr. B**—by repetition of the operations. To remove proteins, fractionation by means of $(NH_4)_2SO_4$ was processed as follows:—

11.1 g. of Fr. B were dissolved in 200 cc. of water (A little insoluble part was centrifuged off; pH of the solution, 6.2), and $(NH_4)_2SO_4$ added till saturation. The voluminous deposit was filtered off after standing overnight and the clear yellowish filtrate was diluted three times and dialyzed for 5 days. SO_4-ions did not entirely disappear, and so after condensation to about 150 cc., the solution was further dialyzed for 2 days when it was perfectly free from SO_4- and NH_4-ions. After condensation to about 10 cc., it was precipitated with 10 volumes of abs. alcohol and some sodium acetate. The precipitate was washed with abs. alcohol and dried as above. 0.70 g. of a white powder was given. Molisch and Bial-Mejbaum positive. Biuret-giving impurities were still detected, though little—**Fr. C.**

0.69 g. of Fr. C was taken up in 20 cc. of water. A somewhat turbid light yellow solution of pH 6.0 was given. 2.8 cc. of a 10% lead acetate solution was added in small portions until no more precipitation. Stood overnight and centrifuged. The clear supernatant fluid was bubbled with H_2S gas. The black precipitate was filtered off and the filtrate (pH 5.8, 30 cc.) was diluted up to 200 cc. and electrodialyzed in a Pauli apparatus through cellophane membranes[3] against changes of water until outer fluids renewed did not undergo any pH shift. Some deposit, that had appeared, was centrifuged off and the supernatant was distilled in vacuo to about 5 cc., precipitated with 10 volumes of abs. alcohol containing some anhydrous sodium acetate. The deposit here was washed with plain abs. alcohol and dried. A white powder weighing 0.23 g. was given—**Fr. D.** Molisch strongly positive; Bial-Mejbaum moderately positive; biuret faintly positive.

The balance (0.22 g.) of this fraction was kneaded with 1 cc. of water. To the solution formed (pH 6.2) were added 11.25 cc. of glacial acetic acid (b.p. 117–118°C, 98%) under shaking. The sticky flocculent precipitate was centrifuged, washed twice with 90% acetic acid and thorough-

ly with ether and dried. 95 mg. of a white powder were obtained—**The Acetic Acid-Insoluble II** (**Ch. II**). To the supernatant fluid were added 3 volumes of ether and the deposit hereby was also washed repeatedly with ether and dried. 103 mg. was this division (a white powder)—**The Acetic Acid-Soluble II** (**Ch. II'**). The former division was Molisch and Bial-Mejbaum positive. Biuret, Neuberg-Saneyoshi, Goldschmiedt, Dische, Osaki-Turumi (the direct as well as the indirect), ninhydrin and Masamune *et al.* (a $HgCl_2$-test[1a]) all negative. Phosphorus, purine and hydrolysable sulfur were not detected. The latter division resembled the former qualitatively, but was faintly biuret-positive.

Properties of the Products

The Acetic Acid-Insoluble I (Ch. I) and II (Ch. II) and the Acetic Acid-Soluble II (Ch. II') were examined. The Acetic Acid-Soluble I (biuret-positive, N 10.9%, hexosamines 6.9%, ash 7.9%) was scarcely examined because it was contaminated with much of protein.

Solubilities

They dissolved perfectly in water, 10% trichloroacetic acid, 90% phenol and the saturated ammonium sulfate, and were not precipitated from water by cupric chloride, lead acetate, barium acetate and baryta. In 90% acetic acid the last one was soluble but the first two were not.

Analyses

The figures obtained are embodied in Table I.

TABLE I

Composition of the Carbohydrate Preparations (Chs. I, II and II')

Analysis	In per cent			In equivalents per equivalent weight		
	Ch. I	Ch. II	Ch. II'	Ch. I	Ch. II	Ch. II'
N*	4.3	3.9	6.1	7.9	6.8	11.8
Hexosamines	20.8†	21.4‡	18.9‡	3.0	2.9	2.8
Galactose§	21.5	23.7	18.9	3.1	3.2	2.8
Mannose§	7.0	7.4	6.7	1.0	1.0	1.0
Pentose as xylose//	6.8	7.1	6.2	1.2	1.1	1.1
L-Fucose¶	8.3	9.6	7.3	1.3	1.4	1.2
Ash**	4.7	2.5	4.8			

* micro Kjeldahl, † Hamasato-Akakura method, ‡ Blix method[6], § Masamune-Ogawa method[7] // Masamune-Ogawa method[8], ¶ Masamune-Ogawa method[9], ** micro Pregl method without use of H_2SO_4.

Optical rotations

The Acetic Acid-Insoluble

$$\text{I:}\quad [\alpha]_D^{25}=\frac{-0.08\times 1}{0.0098\times 1}=-8.1°$$

$$\text{II:}\quad [\alpha]_D^{16}=\frac{-0.13\times 1}{0.0093\times 1}=-13.9°$$

The Acetic Acid-Soluble

$$\text{III:}\quad [\alpha]_D^{15.5}=\frac{-0.23\times 1}{0.0104\times 1}=-22.1°$$

Anti-hemoagglutinative potencies

By our usual procedure (Cf. Oh-Uti[4]) the forces of inhibiting agglutination of A, B and O erythrocytes by normal α and β serum and eel serum respectively were estimated. The results are shown in Table II.

TABLE II

Occurrence of Agglutination of A, B and O Erythrocytes by Human B- and A-serum and Eel Serum respectively after Treatment of the Latter with the Preprations (I, II, I′ and II′)

The B, A and eel serum were so diluted as to have titre 16. ±: faint aggl., +: slight aggl., ++: marked aggl., +++: strong aggl.

Preparation (Ch.)	Group of red cells	Dilution of preparations (1 :)				
		2×10^2	4×10^2	8×10^2	1.6×10^3	3.2×10^3
I	A	++	++	+++	+++	+++
	B	++	++	+++	+++	+++
	O	++	++	++	+++	+++
II	A	++	++	++	+++	+++
	B	±	+	+	+++	+++
	O	++	++	++	++	+++
I′	A	++	++	+++	+++	+++
	B	++	++	+++	+++	+++
	O	++	++	++	++	+++
II′	A	+++	+++	+++	+++	+++
	B	±	+	++	++	+++
	O	++	++	++	++	+++

Paper partition chromatography of the sugar components

The procedure of Masamune and Yosizawa[5] was adapted.

The Acetic Acid-Insoluble I (Ch. I). 40 mg. of the preparation were heated for 5.5 hours with 4 cc. of 1 N H_2SO_4 at 100°C under reflux. The hydrolysate was diluted twice and nearly freed from H_2SO_4 with baryta (Crystals and the saturated solution were added to a pH of 4.6) and condensed to 0.5 cc., of which 0.02 cc. portions were sampled.

The Acetic Acid-Insoluble II (Ch. II). 29 mg. of this preparation were hydrolyzed with 3 cc. of the sulfuric acid solution and after freeing from most of sulfuric acid, condensed to 0.4 cc., and 0.03 cc. portions were

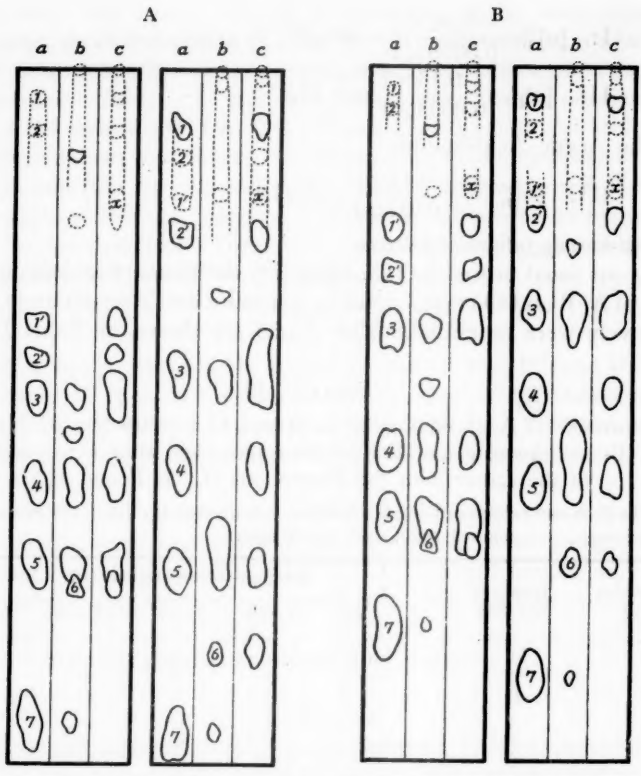


Fig. 1. (A): the chromatograms of Ch. I; (B): the chromatograms of Ch. II. The *n*-butanol-pyridine-water (5:3:2) chromatograms to the left and the *n*-butylacetate-acetic acid-ethanol-water (3:2:1:1) chromatograms to the right in both (A) and (B). Temperature of test: (A), 23–26°C; (B), 20–22°C. Duration of test, 24 hrs. Indicator, aniline hydrogen phthalate of Partridge.

a and b (a 1 *N* H_2SO_4-hydrolysate of traganth): reference runs. c: Ch. I in (A); Ch. II in (B). 1, 1′: chondrosamine sulfate; 2, 2′: glucosamine sulfate; 3: galactose; 4: mannose; 5: xylose; 6: L-fucose; 7: rhamnose.

sampled.

Figs. 1A and 1B are diagrammatic reproductions of the chromatograms demonstrating glucosamine, chondrosamine, galactose, mannose, L-fucose and probable xylose as the sugar components.

Electrophoresis

Chs. I and II were subjected to electrophoresis in a Tiselius apparatus.

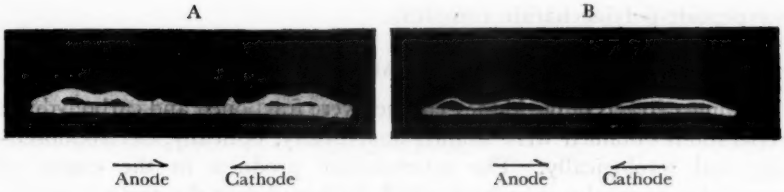


Fig. 2. (A) An electrophoretic pattern of a 1% solution of Ch. I in phosphate buffer, pH 7.8, I=0.29; 14°C; 10 ma; exposure 60 minutes; mobility: the first moving boundary $\frac{1.5\times 0.0117\times 0.3}{0.01\times 3600}=14.6$ $cm^2\cdot volt^{-1}\cdot sec^{-1}\cdot 10^5$, the second moving $\frac{0.9\times 0.0117\times 0.3}{0.01\times 3600}=8.7$ $cm^2\cdot volt^{-1}\cdot sec^{-1}\cdot 10^5$, the third moving $\frac{0.2\times 0.0117\times 0.3}{0.01\times 3600}=1.9$ $cm^2\cdot volt^{-1}\cdot sec^{-1}\cdot 10^5$. (B) A pattern of a 1% solution of Ch. II in carbonate buffer, pH 8.9, I=0.105; 3°C; 10 ma; exposure 30 minutes; mobility: the first moving boundary $\frac{1.0\times 0.00401\times 0.3}{0.01\times 1800}=6.6$ $cm^2\cdot volt^{-1}\cdot sec^{-1}\cdot 10^5$, the second moving $\frac{0.15\times 0.00401\times 0.3}{0.01\times 1800}=1.0$ $cm^2\cdot volt^{-1}\cdot sec^{-1}\cdot 10^5$. Ascending limbs to the left.

The patterns (Figs. 2A and 2B) show three boundaries in the former preparation and two in the latter. The first moving, low boundary of Ch. I appears to represent a contaminating petptide, because Ch. II which contained less nitrogen than Ch. I perfectly or nearly lacked it. The second and third moving components of Ch. I most probably correspond to the first and second moving of Ch. II respectively.

Comments and Discussion

1. Of the fractions of the tissue extract obtained by the aid of 10% NaCl (the first procedure), Fr. I only showed faintly positive Neuberg-Saneyoshi and Goldschmiedt reaction for hexuronic acid, and its subdivisions gave the negative reactions. Hence, pig kidney cortex looks to be very scanty of polyuronic acids.

2. Glucidamins are also believed to be scarce, if present, as were revealed by the experiments on Frs. I, V and VI.

3. The quantitative ratio of the two components represented by the second and third electrophoretic boundaries of Ch. I was the inverse of the corresponding ratio of the corresponding components of Ch. II. Nevertheless, regarding the kinds of sugar component as well as the mutual equivalent proportions of these components in respective substances, the two preparations were quite similar to each other, suggesting that the two electrophoretically different components of either of Chs. I and II were only differently aggregated forms of one and the same carbohydrate

(polypeptide-polysaccharide complex).

SUMMARY

Saline extracts of pig kidney cortices were fractioned and carbohydrate preparations obtained were studied analytically, optically, electrophoretically and serologically. The intermediate products in the course of preparation were also examined which indicated that the tissue is scanty of polyuronic acids and devoid of glucidamins.

Through the Grant Committee for Scientific Researches was given a grant from the Eucation Department in aid to us. H. Masamune.

References

1) Kerr & Seraidarian, T. Biol. Chem., 1949, **180**, 1203.

1a) Masamune, Ogawa & Tsuiki, Tohoku J. Exp. Med., in press. N-glycosides give the negative $HgCl_2$ reaction.

2) Masamune, Tsuiki, Tokutomi & Kikuchi, Tohoku J. Exp. Med., in press.

3) For the use, cellophane membranes were placed in boiling water for 30 minutes and in 5% NaOH at 45°C for 10 minutes in sequence and washed with water.

4) Oh-Uti, Tohoku J. Exp. Med., 1949, **51**, 302.

5) Masamune & Yosizawa, *ibid.*, 1953, **59**, 1.

6) Blix, Acta chem. Scand., 1948, **2**, 467.

7) Masamune & Ogawa, Tohoku, J. Exp. Med., in press.

8) Masamune & Ogawa, *ibid.*, in press.

9) Masamune & Ogawa, *ibid.*, in press.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

A Toxic Substance in Carcinomatous Ascites

Second Report

By

Hirosi Tiba, Isao Takaku and **Genji Kikuchi**
(千葉　博) (高久　功)　(菊池源二)

From the Medico-chemical Institute, Tohoku University, Sendai;
Director: Prof. H. Masamune .

(Received for publication, May 14, 1953)

A further study on the toxic substance in a preceding work[1] revealed that it is 1) heat-unstable, 2) soluble in methanol, ethanol, butanol and acetone, and 3) precipitable from water with lead acetate but not with baryta. It migrated to the anode on electrodialysis as already described by Aikawa.[1] Moreover, an impure preparation of its sodium salt, obtained from an acidic fraction of a carcinomatous ascitic fluid through successive barium and lead salts, analyzed: N 1.8%, amino-N 0.4% and ash 44.6%, suggesting a non-nitrogenous, low-molecular acid for the pure substance. Autopsy findings of the mice killed by injection of the substance (hyperemia and hemorrhage of internal organs) and the effect of it on the beat of a frog heart (depression of the heart) will be also described below, together with some additional observations.

Experimental

1. According to Aikawa, only a part of the toxic substance passes out of the bag when a carcinomatous ascitic fluid is dialyzed, and the remaining migrates to the anode on electrodialysis. The writers substantiated the description of the author as will be exemplified:—850 cc. of a carcinomatous ascitic fluid (Ascitic fluid No. 1. (See Table I)) were distilled in vacuo and dried. The still residue was taken up in 200 cc. of water and dialyzed thoroughly in a cellophane bag against changes of water. The dialysates of pH 8.8–6.8 were condensed as above. The residue here (**Fr. D**) weighed in total 5.5 g. and a mouse, to which 20 mg. of it had been injected subcutaneously per 10 g. bodyweight, showed poisoning symptoms but recovered later. The inner fluid after dialysis was electrodialyzed (in a Pauli apparatus) against changes of water until outer fluids renewed did not undergo any pH change. From the fluids in the anode compartment was recovered 0.42 g. of a substance—**Fr. E.** This fraction killed a mouse in 5 hours after administration of the same dose

TABLE

No. of ascitic fluids	Diagnosis	Colour and transparency	Specific weight	Relative viscosity	Protein content (Esbach) %	Rivalta
1	Stomach cancer, carcinomatous peritonitis	yellow turbid	1018	1.34	4.4	+
2	Biliary liver cirrhosis	yellowish brown, transparent	1014		2.8	−
3	Hepatoma, carc. perit.	hemorrhagic; centrif. supern. liq.: yellow and turbid	1018	1.35	3.9–4.0	+
4	Tuberculous perit.	light yellow	1022	1.88	8.6	+
5	Stom. canc., metastasis in choledochus, carc. perit.	hemor.	1018	1.38	3.8	+
6	Stom. canc., carc. perit.	hemor., slight hemolysis	1018	1.50	4.6–4.8	+
7	Choled. canc., carc. perit.	yellow, translucent	1015	1.09	2.8	±
8	Stom. canc., carc. perit.	very turbid	1015	1.16	5.2	+
9	Cardiac decompensation	light yellow, transp.	1012	1.02		−
10	Stom. canc., carc. perit.	hemor.	1018	1.81	7.6	+
11	Stom. canc., matast. in choled., carc. perit.	yellow, turbid, slight hemor.	1017	1.85	3.9	+
12	Stom. canc., metast. in liver, carc. perit.	yellowish brown, slight hemor.	1013	1.08	2.5	−
13	Stom. canc., carc. perit.	yellow, turbid	1017	1.82	6.8	+
14	Acute yellow liver atrophy	yellow, turbid	1010	1.20		−
15	liver cir.	light yellow transp.	1006	1.08		−
16	liver syphilis	yellow, turbid	1010	1.12		±

Nos. 1–10 of the ascitic fluids are the ones already shown in Table I of Tiba and others'

* Toxicity of a substance is expressed in terms of the dosage in mg. per 10 g. bodyweight the animal died after 12 to 24 hours from injection; ±: the poisoning symptoms occurred

as above. From the inner fluid came out a precipitate during the electrodi-

I

Cell	Volume (cc.)	pH	Yield (g.)				Toxicity of Fr. E (mg.)*
			Fr. D	Fr. E	Cr. Prot. I	Cr. Prot. II	
erythrocyt., leucocyt., cancer cells	850	8.8	5.5	0.42	14	18	20 (+)
	300	8.9	2.5	0.05	0.85	1.2	20 (−)
cancer cells	2,800	8.2–8.4		0.64	24	>38	20 (±)
lymphocyt., leucocyt., few erythrocyt.	1,300	8.0–8.2		0.28	> 6	> 8	20 (±)
many cancer cells, leucocyt., erythrocyt.	6,500	8.6–8.8		1.20	>32	>31	20 (+)
many cancer cells, many erythrocyt., few lympocyt.	2,900	8.8		0.72	>16	>20	20 (+)
cancer cells	2,800	8.8	12	0.62	>10	24	20 (±)
many cancer cells, many leucocyt., erythrocyt.	3,800	8.8		0.98	58	74	20(+) 10(±)
few leucocyt., few lymphocyt.	400	7.8		0.06	>1.2	>1.9	20 (−)
	2,500	8.2		0.58	>10	>15	20 (±)
cancer cells, many leucocyt., many erythrocyt.	4,100	8.2–8.4	28	0.8	30	38	20 (+)
many erythrocyt., many leucocyt.	5,400	7.6–7.8		1.1			20 (+)
leucocyt., erythrocy., lymphocyt.	7,000	8.6		2.3	200	230	20 (±)
a few erythrocyt., a few leucocyt.	1,000	7.6			12	15	
	15,000	8.6			145	155	
	2,700	8.4			15	20	

article.[3]

of a mouse to which it was injected subcutaneously, and the result signed as +, ± or − (+: but the animal did not die; − no poisoning symptoms).

alysis. This—**Prot. II** (18 g.)—and the still residue of the supernatant

fluid—**Prot. I** (14 g.)—both proved nontoxic in 3/2 times the dosage above. Similar fractionation was carried out on 13 ascitic fluids, of which 10 were carcinomatous. The details regarding the properties of those ascitic fluids and their fractions are embodied in Table I.

The dialysates obtained as above contained abundant inorganic salts, which were difficult to eliminate. Hence to avoid this inconvenience, the anodic electrodialysates alone were employed for the following experiments.

2. a) Frs. E's were changed into sodium salts. Fr. E of the carcinomatous ascitic fluids (Nos. 1, 3, 5–8, 10–12) were combined and treated in portions as follows. To cite an example, 0.42 g. of this batch was kneaded with 0.4 cc. of water and 20 cc. of abs. methanol were added. The precipitate was centrifuged, washed with 10 cc. of abs. methanol and dried—Ppt_1 (0.2 g.) Biuret ±, ninhydrin ++, Molisch +. To the supernatant was added 10% NaOH to pH 7.3, whereby again a precipitate occurred. This (Ppt_2) weighed 0.12 g., when dry. Biuret −, ninhydrin ++, Molisch +. The mother fluid was distilled in vacuo to dryness. Yield here 80 mg. Biuret ±, ninhydrin +, Molisch +. Of those three fractions, which were all toxic, Ppt_2 was the strongest and 10 mg. per 10 g. bodyweight killed a mouse within 24 hours after subcutaneous injection. (Similar results were reached by substituting ethanol for methanol.)

In a similar manner 1.44 g. in total of Ppt_2's were obtained, which were put together—**Ppt. II.**

b) The free toxic acid was found soluble in acetone. 0.16 g. of Ppt. II (sodium salts) was dissolved in a small quantity of a dilute HCl solution (pH of the thick solution 1.8) and 20 cc. of acetone were added. The precipitate was centrifuged and washed with acetone. It weighed 30 mg. when dried. The mother fluid was precipitated by addition of abs. methanol containing some NaOH (a watery 10% solution) to pH 7.3, and dried. 50 mg. was the yield. The former precipitate was only faintly toxic (Injection of 20 mg. to a mouse per 10 g. bodyweight exhibited light poisoning symptoms for a short time) but the latter killed an animal within 24 hours in the same dosage.

c) Toxicity of the sodium salt was lowered by heating at 100°C; the free toxic acid was found insoluble in ether. 0.32 g. of Ppt. II was kneaded in a little water and acidified to pH 2.0 with drops of the concentrated HCl and 60 cc. of acetone were added. The precipitate was discarded after centrifugation. The supernatant was distilled in vacuo to a thick solution, then taken up in 20 cc. of water and after filtering off of the insoluble part, dialyzed against changes of water. And the dialysates (pH 2.6–2.0) were distilled together to syrupy consistency and 85% alcohol added. The nearly clear solution was neutralized with 10%

NaOH. The deposit hereby (F_1) weighed 0.14 g. when dried. It killed a mouse within 24 hours in dosage of 10 mg. per 10 g. bodyweight, but after having been heated in physiological saline at 100°C for 5–7 minutes, developed no toxicity even in double the dose.

0.11 g. of F_1 was next dissolved in 2 cc. of water (pH of the solution, 7.4) and after acidification to pH 2.0 with 10% H_2SO_4, shaken with 20 cc. of dry ether. The appearing flocculent precipitate was separated and dried in vacuo over $CaCl_2$. The ethereal solution was dried with anhydrous Na_2SO_4 and precipitated by addition of methanol containing NaOH to pH 7.2. The ether-insoluble (crop, 70 mg.) killed a mouse while the soluble was nontoxic (20 mg. each were injected per 10 g. bodyweight).

3. Ppt. II was transformed into barium salts. 380 mg. of the sodium salts Ppt. II were dissolved in 4 cc. of water and acidified to pH 2.0 with 10% HCl, and 60 cc. of 96% alcohol were added. The deposit was centrifuged off and the supernatant was distilled in vacuo to evaporate off alcohol. The condensate was filled up to 40 cc. with water, the insoluble discarded and the solution stirred up with 1.0 g. of $BaCO_3$ on a boiling water-bath. Centrifuged. The substance, recovered from the centrifugate after removal of barium, proved only faintly poisonous in dosage of 20 mg. per 10 g. bodyweight. To the supernatant (light brown) was added the saturated $Ba(OH)_2$ to pH 8.6 and excess baryta was carried down with CO_2 gas. The solution was then distilled to 2 cc. and precipitated with 20 cc. of abs. alcohol. 80 mg. was the yield here—**Cr. Ppt. A.** It was then taken up in 4 cc. of water and after discarding the insoluble part, precipitated again with 10 cc. of abs. alcohol. 60 mg. of a brown powder were thus given—**Ppt. A** (Ba salts). Lassaigne: N +, S −. Thudicum's H_2SO_4 test for psychosin −, Liebermann-Burchard (for cholesterol) −, Pettenkofer −, ninhydrin +, Millon −, Ehrlich diazo test for histidine +, Sakaguchi +(faint), vanilline-, Hopkins-Cole- and bromine-test for tryptophane −, xanthoprotein −, Molisch + (very weak). Fehling reaction resulted in negative in both the product and its acid hydrolysate. Murexide test for uric acid −; P −. It was readily soluble in water and the solution became dark on standing, suggesting an unsaturated link. 5 mg. per 10 g. bodyweight of it (The substance was injected after removal of barium) killed a mouse in 24 hours. Quantitative analyses: N (micro Kjeldahl) 6.5%, NH_2-N (micro Van Slyke) 2.4%, ash (micro Pregl without use of H_2SO_4) 44.2%.

From the mother fluid of Cr. Ppt. A was recovered a substance by vacuum-distillation, which showed no toxicity in a mouse in dosage of 10 mg. per 10 g. bodyweight (The substance was freed from barium prior to injection).

4. a) Ppt. A (Ba salts) was transformed at first into Pb salts and then

HEALTH CENTER LIBRARY

into sodium salts. 80 mg. of a product corresponding to Ppt. A above were dissolved in 15 cc. of water (A brown solution of pH 7.5 was given) and 35% lead acetate was added until no more precipitate was given (2 cc. of the lead acetate solution were required). Centrifuged. The centrifugate was washed with 4.2% lead acetate and water in turn. Subsequently it was suspended in water, 10% HCl was added to pH 3.5 and H_2S gas was passed. Lead sulfide was filtered off and washed and the filtrate and washing were distilled to about 2 cc.—**Sol. I**—followed by addition of 30 cc. of 96% alcohol. A tiny precipitate appeared on standing the mixture for one day, which was filtered off. The filtrate was neutralized with 10% NaOH and a light-brown precipitate given was washed with alcohol and dried. Yield 45 mg.—**Ppt. B.** It killed a mouse in dosage of 5 mg. per 10 g. bodyweight within 24 hours. Lassaigne for N +, ninhydrin +, biuret −, Ehrlich diazo −, Molisch −. Analyses: N 1.8%, NH_2-N 0.4%, ash (Pregl without use of H_2SO_4) 44.6%.

The mother fluid of the lead acetate-precipitate (pH 5.6) was freed from Ba and Pb by addition of 4 *N* H_2SO_4 and further bubbling with H_2S and distilled in vacuo to about 1 cc. (pH of this condensate 4.6) and 20 cc. of 96% alcohol added. The deposit was centrifuged off and to the supernatant was added 10% NaOH to pH 7.1. The deposit here amounted to 16 mg. when dry. 10 mg. of it killed a mouse within 24 hours, when injected to a 10 g. mouse.

b) Formaldehyde did not impair the toxicity of the substance in question. 20 mg. of a product like Ppt. B above were dissolved in water and a dilute formaldehyde solution (neutralized) was added drop by drop with following neutralization until the solution gave no more ninhydrin reaction (At this stage the solution contained no excess formaldehyde, because $AgNO_3$-NH_4OH test resulted in negative). The solution was distilled in vacuo to dryness. The residue (12 mg.) possessed the same toxicity as the ninhydrin positive material used, namely 5 mg. of it per 10 g. bodyweight killed a mouse within 24 hours.

c) The toxic substance in the form of free acid is soluble in butanol. A solution like Sol. I in a) was distilled in vacuo to dryness. The still-residue dissolved in butanol.

5. Autopsy findings. a) In mice which died in less than 20 minutes after injection of a large amount of the poison, no perceptible changes were observed in internal organs.

b) 5 mice, which died either after 24–48 hours from a single injection of a small amount of the poison or after repeated injections (once a day) of a less amount, underwent remarkable pathological changes in internal organs as follows.

a) In 3 mice, liver, kidney and spleen showed extreme hypertrophy

and dark red appearance, and 2 of those animals excreted bloody urine. Microscopic findings:— Liver: general hyperemia, hemorrhagic spots, changes like fatty degeneration at several parts, no necrosis; kidney: strong hyperemia, hemorrhage, almost no change in glomeruli and tubelets; spleen: hyperemia, hemorrhage.

β) The other 2 animals underwent the changes above in liver and spleen (Evident were hyperemia and hemorrage), but not in kidney.

6. Action on a frog heart. 30 mg. of Ppt. II (which killed a mouse in 5–12 hours after injection of 20 mg. per 10 g. bodyweight) were dissolved in 3 cc. of water and diluted with Ringer solution. The tracings of a frog heart perfused with the solutions of different concentrations are reproduced in Fig. 1. A 0.2% solution depressed the systole and diastole to an enormous extent immediately, and even a 0.01% solution proved effective, but a 0.001% solution was quite inert.

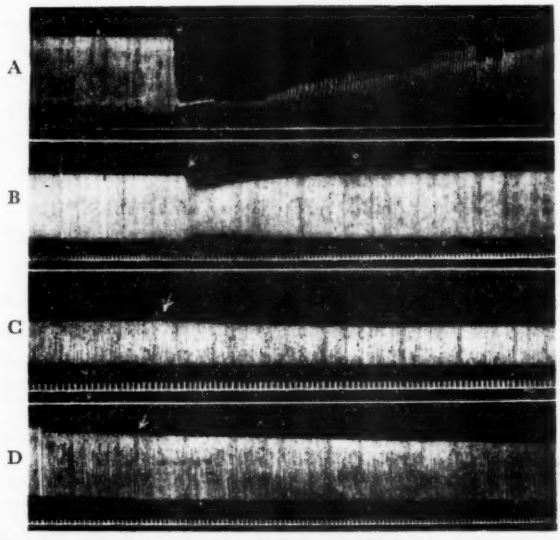


Fig. 1. A frog heart perfused with solutions of Ppt. II. At ↓ was given a solution (a 0.2, 0.1, 0.01 and 0.001% solution respectively in A, B, C and D). Upstroke represents systole.

Through the Grant Committee for Scientific Researches was given a grant from the Education Department in aid to us. H. Masamune.

References

1) Aikawa, Tohoku J. Exp. Med., 1949, **51**, 351.
2) pH of the fluid 4.5.
3) Tiba, Takaku and Satoh, Tohoku J. Exp. Med., 1951, **53**, 223.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

On a Protein of Ascites

Second Report*

By

Hajime Masamune, Hirosi Tiba, Isao Takaku,
(正宗 一) (千葉 博) (高久 功)
Genji Kikuchi and **Yoiti Satoh**
(菊池 源二) (佐藤 與一)

From the Medico-chemical Institute, Tohoku University, Sendai

(Received for publication, May 14, 1953)

The protein of ascitic fluids named Prot. I in a former work,[1] which are unprecipitable by electrodialysis, were further studied. In this account, several properties particularly the serological specificity of the protein in the carcinomatous ascitic fluids with primary tumor in stomach will be described in contrast to those in non-cancerous ascitic fluids.

Experiments and Comments

Materials

Crude **Prot. I** of the carcinomatous ascitic fluids with primary seat in stomach and the corresponding crude protein (**Prot. I'**) of the non-cancerous exclusive of No. 15, embodied in Table I of the just preceding article,[2] were combined separately and purified by repeated electrodialysis as already reported.[1] From 330 and 30 g. of the crude batches, 150 and 16 g. respectively of the purified products were obtained.

Properties

1. Electrophoresis (Fig. 1). The products gave only a single boundary after electrophoresis in phosphate buffer, pH 7.8 (current 10 ma; temperature 10°C in the case of the carinomatous protein and 12°C in that of the non-cancerous). Mobilities of the boundaries:

The carcinomatous protein, $10.0\ cm^2 \cdot volt^{-1} \cdot sec^{-1} \cdot 10^5$
The non-canc. protein, 8.1 "

2. Heat coagulation. 10–15 mg. each of them were dissolved in 5 cc. of water, acidified to pH 4.5 with acetic acid and placed in a boiling water-bath. They did not coagulate even after 30 minutes, but precipitated on addition of a little NaCl. The heating was continued for further

* The 9th report of Masamune and coworkers' "On Proteins and Amino Acids."

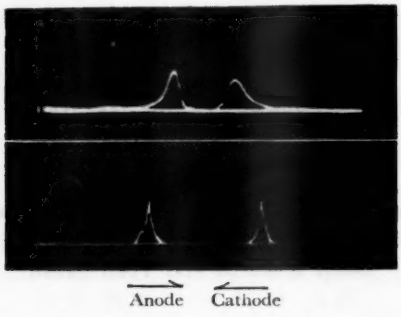


Fig. 1. Electrophoretic patterns of a 1% solution of (A) Prot. I and (B) Prot. I′ in phosphate buffer, pH 7.8, I=0.29; 10° and 12°C respectively; 10 ma; exposure 30 minutes. Mobility of the boundaries:

Prot. I.: $\frac{1.35\times 0.0089\times 0.3}{0.01\times 3600}=10.0$ cm^2·volt^{-1}·sec^{-1}·10^5

Prot. I′: $\frac{1.1\times 0.0088\times 0.3}{0.01\times 3600}=8.1$ "

Ascending limbs to the left.

10 minutes and the precipitate centrifuged off. The supernatant fluid showed faintly positive biuret reaction but negative Molisch.

3. Acid precipitation. Picric, trichloroacetic and sulfosalicylic acid precipitated the proteins as already reported.

4. Test-tube tests. Strong biuret and moderate Molisch reaction were given. Tryptophane, histidine, tyrosine and a trace of phosphorus were detected, but bile pigments and bile acids not.

5. Analyses. The product from the carcinomatous ascitic fluids: N (micro Kjeldahl) 15.0%, hexosamines (Hamasato-Akakura) 0.6%, ash (Pregl) 0.01%. The product from the non-cancerous ascitic fluids: N 15.3%, hexosamines 0.5%, ash none.

6. Specific refraction. 20 mg. each of them were dissolved in 2 cc. of water (pH of the solutions, 5.5) and the refraction measured in an Abbe refractometer. Temperature of test 17.5°C.

The carc. protein: $\frac{(1.33481)^2-1}{(1.33481)^2+2}\times\frac{1}{1.0026}\times\frac{100}{1}-\frac{(1.33320)^2-1}{(1.33320)^2+2}\times\frac{1}{0.999}\times\frac{100-1}{1}=0.225°$

The non-canc. protein: $\frac{(1.33485)^2-1}{(1.33485)^2+2}\times\frac{1}{1.0030}\times\frac{100}{1}-\frac{(1.33320)^2-1}{(1.33320)^2+2}\times\frac{1}{0.999}\times\frac{100-1}{1}=0.217°$

7. Acid dye-combining forces. a) 10.03 and 10.04 mg. respectively of the carcinomatous and non-cancerous proteins were taken up in 10 cc. of 0.02 *N* HCl and titrated with a 0.1% solution of Tropeolin O (air dry) until no more precipitation. 5.84 and 5.96 cc. of the dye solution were

used. Namely, 1 mg. of the proteins combined with 0.58 and 0.59 mg. respectively of the dye. b) Titration with congo red (a 0.1% solution). The proteins were titrated similar to above. The carcinomatous combined with 0.12 mg. of the dye per mg. and the non-cancerous 0.13 mg. of the dye.

Thus the findings above (1.–7.) indicated that the two kinds of protein were similar very closely.

8. Preliminary experiments regarding the specific determinant group of the carcinomatous protein. In one of the foregoing reports,[1] it was mentioned that the carcinomatous protein produces in a rabbit an antibody specific to this protein. Here to decide whether the amino acid grouping or the carbohydrate moiety possesses this specificity, at first portions of the protein, condensed with formaldehyde, digested with trypsin or denatured by heating, were tested as to their potency of giving precipitin reaction with an immune serum of the untreated protein.

a) Treatment of the protein with formaldehyde. 50 mg. of the protein were taken up in 5 cc. of water (pH of the solution 5.8) and after addition of 0.1 cc. of formalin, adjusted to neutral with 1% Na_2CO_3. The mixture was stood at room temperature (16°C) for 1 hour and precipitated with 35 cc. of alcohol containing some NaCl and centrifuged. The centrifugate was washed with alcohol and dried in vacuo over $CaCl_2$. Yield 50 mg.

b) Tryptic digestion. 20 mg. of the protein in 2 cc. of water and 20 mg. of Kahlbaum trypsin in 2 cc. of water were mixed together, alkalinized to pH 8.5 with 1 *N* Na_2CO_3 and stood in a thermostat at 37°C (Toluene was used as a preservative), readjusting the pH to the original with the Na_2CO_3 solution from time to time. After 7 days no change of pH occurred anymore (The alkali requisite for the readjustment was 1.5 cc. in total). Toluene was aerated off. Then the solution was neutralized with 1% HCl and filled up to 10 cc. with water.

c) Heating. 20 mg. of the protein were taken up in 10 cc. of water, acidulated (to pH 4.4) with 20% acetic acid and heated in a boiling water-bath for 30 minutes. Then it was precipitated and washed with alcohol as in a) and dried. For the precipitin assay the substance given was dissolved in water by neutralization with NaOH and an equal volume of 1.7% NaCl was added.

d) Precipitin reaction. As is illustrated in Table I, the potency of the protein of giving the precipitin reaction was lowered by the treatments above. But the decreases in case of the reaction with the absorbed serum were so small that the determinant group appeared to reside in the tiny carbohydrate moiety but not in the amino acid grouping.

9. Separation of the carbohydrates from the different proteins.

TABLE I

Precipitin Reaction of Portions of Prot. I of the Carcinomatous Ascitic Fluids originating in Stomach Cancer, treated and untreated, with a Rabbit Serum Immunized with the Untreated Protein

Portion of Prot. I	Treatment of the serum*	Dilution of substances (in physiol. saline) (1 :) .						
		10^3	10^4	10^5	10^6	10^7	10^8	10^9
Untreated	unabsorbed	+	+	+	+	+	±	−
	absorbed	+	+	+	±	−	−	−
Treated w. formalin	unabsorbed	+	+	+	±	−	−	
	absorbed	+	+	±	−	−	−	
Digested w. trypsin†	unabsorbed	+	+	+	±	−	−	
	absorbed	+	+	+	±	−	−	−
Heat-denatured	unabsorbed	+	+	+	±	−		
	absorbed	+	+	±	−	−		

* The absorption was effected with Prot. I′ (united). † Trypsin showed the positive precipitin reaction with the unabsorbed serum at a dilution of $1:10^2$ and the mixture of equal amounts of trypsin and the untreated Prot. I at a similar dilution as the solution of the latter alone with respect to the protein.

a) *First stage.* 120 g. of the carcinomatous protein batch were digested with 10 volumes of 1 *N* NaOH for 3 days at room temperature (8–15°C). Then 30% acetic acid was added, under cooling in water, to pH 4.6 and the precipitate occurring was discarded after centrifugation. The supernatant was dialyzed against running water for 3 days. Some precipitate hereby was also rejected. The fluid was distilled in vacuo to about 200 cc., and acidified to pH 4.6 with acetic acid, whereby again a precipitate occurred. The centrifuged supernatant was concentrated to a syrup and about 5 volumes of abs. alcohol added. The deposit was washed with alcohol and dried. 20 g. of a yellowish powder were given.

Second stage. It was dissolved in 40 cc. of water and 40 cc. of 10% trichloroacetic acid were added. The precipitate was separated off by means of a centrifuge and the slightly turbid supernatant was brought to pH 4.6 with sodium acetate, followed by addition of 8 volumes of abs. alcohol. The precipitate here was washed with alcohol and dried. The trichloroacetic acid-precipitable was eliminated as far as possible by repetition of the process. And 8 g. of the trichloroacetic acid-unprecipitable (a white powder) were obtained.

Third stage. 7.5 g. of it were kneaded with a little water and shaken with 200 cc. of 98% acetic acid. The insoluble part was washed with alcohol and dried. The operation was conducted over again. The glacial acetic acid-insoluble (a white powder) amounted to about 2 g.

Fourth stage. It was dissolved in 40 cc. of water (A light-yellow so-

lution of pH 5.6 was given; the insoluble was rejected), and 4 cc. of 10% lead acetate were added. The centrifuged supernatant was bubbled with H_2S-gas and filtered. The filtrate, which was light-brown colored, was stirred up with 2 g. of purified animal charcoal, stood overnight in a thermostat at 37°C, and filtered again. The non-colored, transparent solution obtained was exhausted with ether (pH of the solution rose thereby to 5.6), distilled in vacuo to a syrup and dehydrated and washed with abs. alcohol. The product dried—**Ch. I**—amounted to about 0.2 g.

b) With the aim particularly, Prot.-I′ of ascites No. 15 (liver cirrhosis) was purified, and 60 g. were cleft and fractioned similar to above. 90 mg. of a substance corresponding to the final product in a) were obtained—**Ch. I′**.

c) Properties of the carbohydrate (amino acid-polysaccharide complex) preparations, i) *Test-tube tests*. The two preparations gave coincident consequences: biuret, ninhydrin, Millon, Ehrlich diazo (for histidine) and Neuberg-Saneyoshi (for uronic acid) all negative. Phosphorus and purine not detected. Molisch strongly positive (A dark violet ring was given).

ii) *Paper partition chromatography of the sugar components*. 30 mg. of each of them were hydrolyzed with 3 cc. of 1 *N* H_2SO_4, by heating under reflux at 100°C for 5.5 hours, and after eliminating most of H_2SO_4 with baryta (pH of the ensuing solution 4.6), condensed to about 0.5 cc., of which a 0.04 cc. portion was sampled on a strip of filter paper for the chromatography after Masamune and Yosizawa.[3]

As Figs. 2A and 2B reveal, both of the carbohydrates were found to contain glucosamine, chondrosamine, galactose, mannose and L-fucose as the sugar components.

In passing it might be worthwhile to mention that ascitic fluids cannot be simple filtrates of blood serum, because in the serum there exists no protein possessing a carbohydrate of such composition as those here (Cf. Inazawa[4] and the literature cited there, and Masamune & Yosizawa[4a]).

iii) *Analyses and optical rotations*. The figures obtained are embodied in Table II. The carbohydrate from the carcinomatous protein (Ch. I) was poorer in nitrogen than that from the non-cancerous (Ch. I′), but both contained total amino sugars and total nonamino sugars in similar equivalent ratios. The former Ch. was more levorotatory than the latter probably because of the more proper-polysaccharide content.

iv) *Electrophoresis*. As are seen in the patterns in Figs. 3A and 3B, the carbohydrates gave each two boundaries. It is not precluded that the proteins contain two polysaccharides, but more probably one polysaccharide, which is the sole one, was disintegrated during separation, giving rise to fragments moving at various rates, or its molecules were separated as conjugated entities with respective peptide chains of different lengths.

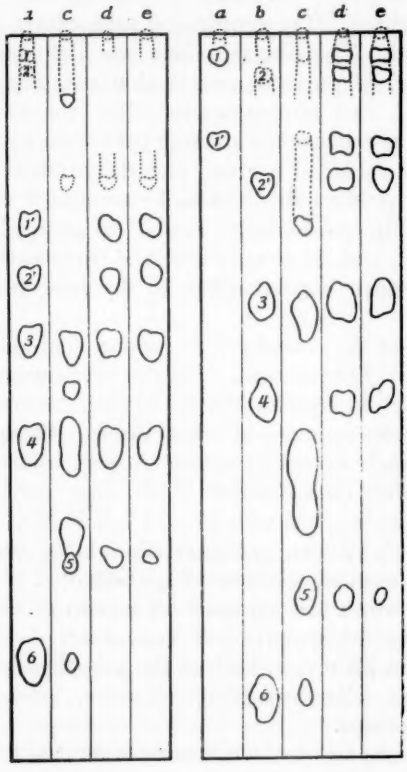


Fig. 2. Diagrammatical reproduction of (A) *n*-butanol-pyridine-water (5:3:2)- and (B) *n*-butylacetate-acetic acid-ethanol-water (3:2:1:1)-chromatograms developed with aniline hydrogen phthalate of Partridge. Duration of test 24 hrs.; temperature of test 23–26°C.

a, b and c (a 1 *N* H_2SO_4-hydrolysate of traganth): reference runs; d: Ch. I; e: Ch. I'. 1 and 1': chondrosamine sulfate; 2 and 2': glucosamine sulfate; 3: galactose; 4: mannose; 5: L-fucose; 6: rhamnose.

v) *Precipitin reaction.* The rabbit antiserum of the carcinomatous protein (the same immune serum as employed above) showed precipitin reaction with Chs. I and I' as is shown in Table III. Ch. I was not so potent to give the reaction with the absorbed immune serum as had been expected from the results in Table I and the poor carbohydrate content of the protein. But it may be ascribed to the alkaline procedure of preparation which is probable to have partially cleft the carbohydrate molecules (Cf. Masamune *et al.*[5]). Thus the writers incline to believe

TABLE II

Composition and Optical Rotation of the Carbohydrate of Prots. I and II

	Ch. I	Ch. I′	Ch. I	Ch. I′
Analysis				
	In per cent		In equivalents per equivalent weight	
N*	2.3	3.0	1.2	1.8
Hexosamines†	24.3	21.3	1.0	1.0
Non-amino sugars as mannose				
Indole reaction	76.2	62.9	3.1	2.9
Orcinol r.	59.7	47.4	2.4	2.2
Ash‡	6.2	8.3		
Rotation				
$[\alpha]_D$ in water (°)	−46.0	−35.4		

* Micro Kjeldahl † Hamasato-Akakura method. ‡ Pregl method without use of H_2SO_4.

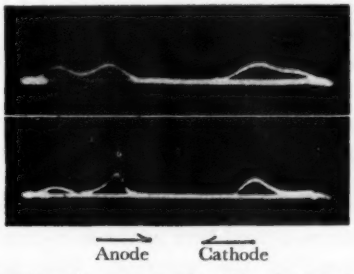


Fig. 3. Electrophoretic patterns of a 1% solution of (A) Ch. I and (B) Ch. I′ in carbonate buffer, pH 8.9, I=0.105; 10°C; 10 ma; exposure 30 minutes. Mobility of the boundaries:

Ch. I, i: $\frac{0.75\times0.00645\times0.3}{0.01\times1800}=8.1$ $cm^2\cdot volt^{-1}\cdot sec^{-1}\cdot10^5$

ii: $\frac{0.05\times0.00645\times0.3}{0.01\times1800}=0.5$,,

Ch. I′, i: $\frac{0.8\times0.00594\times0.3}{0.01\times1800}=7.9$,,

ii: $\frac{0.07\times0.00594\times0.3}{0.01\times1800}=0.7$,,

(i: first moving boundary, ii: the second moving.)
Ascending limbs to the left.

that the carbohydrate moiety possesses the specific determinant structure of the carcinomatous protein.

TABLE III

Precipitin Reaction of Chs. I and I′ with the Antiserum of Prot. I

Ch.	Treatment of the immune serum*	Dilution of Chs. (in physiol. saline) (1 :)					
		10^2	10^3	10^4	10^5	10^6	10^7
I	unabsorbed	+	+	+	+	−	−
	absorbed	+	+	+	−	−	−
I′	unabsorbed	+	+	−	−	−	−
	absorbed	−	−				

* The absorption was effected with Prot. I′ united.

SUMMARY

1. The carcinomatous ascitic fluids with primary tumor in stomach and the non-cancerous were fractioned, and the proteins remaining in solution on electrodialysis were purified from the crude products combined separately according to the carcinomatous or non-cancerous nature of the fluids.

2. The carcinomatous and the non-cancerous protein (preparations) both proved homogeneous electrophoretically and showed no noteworthy difference in heat-coagulability, precipitability by alkaloid precipitants, chemical qualitative reactions, analyses, specific refraction and acid dye combination.

3. The former protein underwent almost no alteration in reactivity (precipitin reaction) with its antiserum, absorbed with the latter protein, by tryptic digestion, treatment with formaldehyde and heating.

4. The carbohydrate moiety of the two proteins (The protein from a liver-cirrhotic ascites was used here as the non-cancerous) were separated after alkaline cleavage of the proteins. The preparations resembled each other qualitatively (test-tube test and paper partition chromatography of the sugar components), quantitatively (The one from the carcinomatous protein contained somewhat less amino acids) and electrophoretically.

5. The carbohydrate preparation from the carcinomatous protein proved to act as the determinant group specific to that protein by the precipitin reaction with an immune serum of that protein, absorbed with the non-cancerous protein.

6. Judging from kinds of sugar component of the carbohydrate preparations, it is plausibly claimed that ascitic fluids can not be mere filtrates of blood serum.

Through the Grant Committee for Scientific Researches was given a grant from the Education Department in aid to us. H. Masamune.

References

1) Tiba, Takaku & Satoh, Tohoku J. Exp. Med., 1951, **53**, 223.
2) Tiba, Takaku & Kikuchi, *ibid.*, this volume, p. 77.
3) Masamune & Yosizawa, *ibid.*, this volume, p. 1.
4) Inazawa, *ibid.*, 1951, **54**, 53.
4a) Masamune & Yosizawa, *ibid.*, 1950, **53**, 155.
5) Masamune, Yosizawa & Masukawa, *ibid.*, 1953, **48**, 381.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

Clinical Experiments of Orally Administering Therapy of Casein and Defatted Soya-Bean Hydrolysates

By

Shinichi Saito
(齋藤 信一)

From the Medical Clinic of Prof. Kurokawa, Tohoku University, Sendai

(Received for publication June 12, 1953)

We may easily suppose that various kinds of nutrients which have been decomposed into the form in which they are just going to be absorbed will permeate into the blood vessels through intestinal walls with no heavy burden. Co Tui *et al.*[1] were the first who from this point of view employed amino acids, the final compost of protein, for patients as protein supplement of the body. Since then clinical utilization of hyperalimentation treatment with amino acids has been tried by many investigators, among whom was Nathan Steinberg[2] who used soya-bean hydrolysed with ferment for this purpose. Mostly used as animal amino acids is ferment-hydrolysed casein which componentally contains much of essential amino acids and as vegetable amino acids soya-beans. But the former is very expensive and when it is of thick density unpalatable. If such amino acids as ferment-hydrolysed soya-bean, very cheap and not so unpalatable, are effectively used, it is most desirable. Examining vicissitude of symptoms, body weight, nitrogen balance, excretion rate of nitrogen in urine and other necessary items, I studied whether amino acids obtained by hydrolysing defatted soya-beans might be of service for the same purpose.

Experimental

During 2 years and 4 months of my experiment I treated 15 cases, but one case of them from the unsatisfactory collaboration on the part of the patient and one case from the selfishness of the patient could not produce reliable results. The other 13 reached significant conclusions.

Methods

The diet for the patients contained protein accurately calculated. It had a high calory within the limit possible. Furthermore, casein ferment-hydrolysate, defatted soya-bean ferment-hydrolysate and a mixture

of the both were given, sometimes with maltose, sometimes not, to the patients. In this case 100 to 20 g. of the mixture, which had been diluted in 500 to 200 cc. of water, was put on about two weeks for evacuation every day.

The analysis of the ingredients of the mixed amino acids by paper chromatography revealed casein amino acids to contain arginine, histidine, lysine, tryptophan, phenylalanine, tyrosine, methionine, cystine,leucine, isoleucine, valine, glycine, serine, proline, alanine, aspartic acid, glutamic acid, etc. and defatted soya-bean amino acids to contain the foregoing items except arginine, histidine, tryptophan, phenylalanine, besides asparagin. To examine nitrogen balance, the whole stool and urine the patient excreted were weighed every day.

The stool was homogenously stirred, part of which was taken out, dried, and reduced to powder in a mortar. Further, quantitative analysis of nitrogen was made of a part of the powdered stool, and then on the basis of the quantity of nitrogen thus obtained the quantity of nitrogen contained in the whole stool was calculated. The same process was carried on with urine. Quantitative analysis of nitrogen was made of part of homogenously churned urine, from the result of which was calculated the quantity of nitrogen contained in the whole urine. Kjehldhahl's method was followed. In the numerical calculation of nitrogen balance the accustomed method was pursued, that is, the calculation was made by subtracting the excreted nitrogen from that which had been given. Here trifle nitrogen containers, such as epidermis metabolite, nasal secrete, sweat, hair, etc. were excluded. To know the vicissitude of symptoms a card on which a number of main symptoms was written had been handed to each patient who could be made to put on it the four marks (−) (+) (++) (+++) according to his conditions, so that a synthetic judgement, whether deterioration or improvement, might be brought.

When the three kinds of amino acids were given to patients, the dicision which had a better absorption rate of nitrogen was thus made. The nitrogen excreted in the urine of those patients whose nitrogen balance was kept positive was divided by the nitrogen given to them.

This is an excretion rate of nitrogen in urine, and when this rate was low the absorption was judged as good.

Further examination was conducted as to the variation of weight, change of nonprotein nitrogen remaining in the blood, quantity of serum protein and so on.

During about 17 days when amino acid solution was orally administered, several days before the treatment included, besides the diet given by me only such fruit as contained no or little calory and protein was allowed to give. Various vitamins were sufficiently supplemented through

TABLE 1

No.	Disease	Name	Sex	Age	Casein amino acid (g.)	No. of days	Defatted soya-bean amino acid (g.)	No. of days	Both mixed amino acid (g.)	No. of days	Nitrogen remaining in body after treatment (g.)	Body weight gained after treatment (kg.)	Excretion rate of nitrogen in urine				Serum protein before treatment (g/dl)	Serum protein after treatment (g/dl)	n. p. n. before treatment (mg/dl)	n. p. n. after treatment (mg/dl)
													Without amino acid (%)	Casein amino acid (%)	Defatted soya-bean a.a. (%)	Both mixed a.a. (%)				
1	Gastric ulcer	T.T.	♂	49	1400	14					39.52	2.0					7.1	8.8	39	47
2	Gastric ulcer	T.I.	♂	29	1400	14					19.81	2.0					7.0	7.0	29	39
3	Gastroptosis	F.H.	♀	29	878	13					11.74	0.7							23	34
4	Afebrile pulmonary tuberculosis	S.S.	♀	40	1040	13					27.29	0.8					8.0	7.2	26	32
5	Hypertrophic cirrhosis of the liver	N.K.	♀	47	653.3	14					10.29	1.3					6.6	7.2		
6	Afebrile pulmonary tuberculosis	S.S.	♀	40			700	10			14.9	0.4								
7	Severe cirrhosis of the liver	S.S.	♂	23			244	6	200	4	6.91	[redacted]					7.0	7.2	31	
8	Gastric ulcer	F.C.	♂	28			430	8	360	6	Ascites −25.28 3.35	[redacted] 0.4					6.8	7.4		
9	Acute hepatitis	T.O.	♂	24	40	2	410	7	240	4	30.72	−1.4					7.2	8.0	26	40
10	Dyschezia	T.M.	♂	23	150	4	160	4	120	4	48.90	1.1	71.6	61.4	71.5	88.9	7.8	7.4	37	32
11	Subacute enterocolitis	S.S.	♀	26	51.1	3	84	5	59.6	3	9.227	−1.0	87.5	63.1	83.6	69.1	6.8	7.8	36	42
12	Progressive muscular dystrophy	T.K.	♂	22	110	3	120	3	110	3	14.65	0.6	81.90	71.4	76.8	75.4	8.0	7.8	21	29
13	Chronic colitis	H.C.	♀	42	150	5	120	4	120	4	17.20	0.2	90.5	73.4	81.0	53.9	7.4	6.8	23	25

Remarks: 1) The number of gram of amino acid is to be converted into the weight of nitrogen by multiplying it by 0.118 in case of casein and by 0.0826 in case of defatted soya-beans. 2) The ratio of mixed amino acid is not certain. Therefore more precise calculation than that given in the above table is impossible.

the skin. Subject, amount of amino acid administered, total number of nitrogen balance, absorption rate etc. were tabulated into Table I.

Results

When only casein hydrolysate was administered 4 cases out of 5, from Case 1 to Case 5 in Table I, showed comparatively rapid increase in weight and bodily retention of nitrogen, excluding Case 3 of gastroptosis. Variation of physical strength was presumed by grip strength, and no case of decrease was found. Conditions of Case 5 of cirrhosis of the liver are shown in Table II below.

TABLE II

A Case of Cirrhosis of the Liver

Date	Casein amino acid (g.)	Maltose (g.)	Calories (Cal.)	Nitrogen given in total (g.)	Nitrogen balance	Grip strength (kg.)	Body weight (kg.)
11/Aug.	80.0	80.0	2966	20.64	−0.172	16	63.2
12	80.0	80.0	2966	20.64	−0.446	15	63.2
13	26.66	26.66	2555	12.74	−1.501	16	63.3
14	26.66	26.66	2555	12.74	−2.644	16	63.3
15	30.00	30.00	2581	14.74	−0.670	10	63.3
16	30.00	30.00	2581	14.74	+0.266	12	63.3
17	40.0	40.00	2658	15.92	−0.931	10	64.0
18	40.0	40.0	2658	15.92	+1.335	15	64.0
19	50.0	50.0	2735	17.10	−0.239	14	64.3
20	50.0	50.0	2735	17.10	+2.820	12	64.0
21	50.0	50.0	2735	17.10	+1.654	14	64.9
22	50.0	50.0	2735	17.10	+3.148	16	64.1
23	50.0	50.0	2735	17.10	+2.822	12	64.2
24	50.0	50.0	2735	17.10	+4.931	14	64.4

Remarks: 1) N. given in 14 days; 230.68 g. 2) N. excreted in urine; 176.94 g. 3) N. excreted in stool; 43.37 g. 4) Total N. balance +10.37. 5) Increased body weight; ca 1.2 kg.

Administration of only defatted soya-bean hydrolysate served little for weight increase, though positive nitrogen balance was maintained.

In the 4 cases where the three kinds of amino acid were administered to each patient, bodily absorption rate of nitrogen was lowest in the case administered with defatted soya-bean hydrolysate if the rate is inversely proportional to the excretion rate of nitrogen in urine. One of the cases is given as Table III. Now I shall state about changes of symptoms. In the two cases of gastric ulcer for which casein hydrolysate was employed niches required almost as many days to disappear as in case of spontaneous

TABLE III

A Case of Progressive Muscular Dystrophy

Date	Casein amino acid (g.)	Defatted soya-bean amino acid (g.)	Calories (Cal.)	Nitrogen given (g.)	Nitrogen balance	Grip strength (kg.)	Body weight (kg.)
19/Sept.	0	0	2113	12.5	−1.305	50	49.9
20	0	0	2113	12.5	+2.220	50	49.5
21	0	0	2113	12.5	−1.947	50	49.5
22	20	0	2167	14.86	+1.326	50	49.4
23	30	0	2195	16.04	+1.804	51	49.0
24	30	0	2195	16.04	+1.184	51	49.2
25	30	0	2195	16.04	+1.137	50	49.2
26	10	10	2146	14.50	−1.348	50	49.9
27	15	15	2169	15.50	+2.383	50	49.6
28	15	15	2169	15.50	+1.517	51	49.6
29	15	15	2169	15.50	+3.604	50	50.1
30	0	30	2170	14.97	+0.289	50	50.2
1/Oct.	0	30	2170	14.97	+0.837	50	50.1
2	0	30	2170	14.97	+0.849	50	50.2
3	0	30	2170	14.97	+1.105	52	50.2

Remarks:

1) Average excretion rate of N in urine:
   a) from 19/Sept. to 21/Sept. 81.90%
   b) from 22/Sept. to 25/Sept. 71.43%
   c) from 26/Sept. to 29/Sept. 75.41%
   d) from 30/Sept. to 3/Oct. 76. 81%

2) a) N. given in 15 days 22.42 g.
   b) N. excreted in urine 164.63 g.
   c) N. excreted in stool 39.13 g.
   d) Total N. balance +17.66
   e) Body weight increased in 15 days by 0.6 kg.

cure. Of gastroptosis I had one case where the condition was thought to have become reactive to strychinine preparations. In the case of cirrhosis of the liver edema in the skin went down remarkably, besides the body weight increased without ascites. No particular effect was observed on pulmonary tuberculosis.

Generally speaking, at the beginning of this treatment unpalatability of casein amino acid caused the loss of appetite for average three days, but it recovered on the fourth day.

With defatted soya-bean hydrolysate I could not see any apparently improved case, though it has an advantage of not unpalatability. Mixed amino acid solution came next to casein amino acid in effectiveness.

In the use of defatted soya-bean amino acids an unexpected effect was achieved on a case of dyschezia. These amino acids were so efficacious as a laxative that a patient who had defecated once in ten days came to

evacuate soft stool once every day, maintaining positive nitrogen balance. Needless to say, casein amino acids are of better service as an aperient medicine.

In the case of chronic colitis on which constipation has ill effects, the unwellness felt in the part of hypogastrium was diminished by using amino acid.

Nonprotein nitrogen in the blood of the patients who were under the treatment with amino acid increased in 8 cases out of 9, and serum protein increased in quantity in 6 cases out of 11; one unchanged and 4 decreased.

Sufferers from gastric ulcer with hyperacidity were given 500 cc. of casein amino acid solution (pH 5.6) a day, 100 cc. for one time, and gastric juice was examined after 30 minutes. In one case was hyperacid and the other normoacid.

Comment

We have two ways of decomposing protein artificially—decomposition with acid and decomposition with ferment.

If amino acid is produced by decomposing protein with acid the salt which is also produced then makes the amino acid very difficult to take out in its pure form. Moreover, this salt irritates the wall of the stomach, or urges the secretion of gastric juice, sometimes causing catarrh. Therefore the amino acid to be used for medical treatment must be the one decomposed with ferment. For this reason I made my experiment with this sort of amino acids and recognized the following points.

In the oral administration of amino acids the difference of materials influences utilization. Absorption rate through intestinal canals is thought much higher in the form of amino acid than in that of source material. The density of amino acid solution seems best when it makes the stool of its users slightly soft. Taking too much of it is apt to be a cause of diarrhea.

To sufferers from hepatitis for which effects of methionine can be expected, even defatted soya-bean amino acids may bring satisfactory results, if they can take it from their mouth.

The vigor of patients does not always increase parallel with the increase of body weight, but nitrogen balance must always be positive. Increases of body weight seem to be influenced by the amount of such amino acids as lysine, tryptophan, histidine, etc. which bear a close relation to the increased weight, as well as by the maintenance of positive nitrogn balance. Furthermore, difference between casein amino acids and defatted soya-bean amino acids in nitrogen supplement is related to the nitrogen content in the same weight. The nitrogen content ratio of casein

amino acids I used to defatted soya-bean amino acids is 1:0.7.

From a half to a third of the protein supplied to the patients was given in the form of amino acid. I think it is best that the whole amount of protein to be supplied should always be about 90 g.

Donald[3] is of opinion that amino acid administering therapy is not so beneficial, but, though defatted soya-bean amino acids maintain only positive nitrogen balance and vigor, casein amino acids make up the protein of body well and are very advantageous to those patients who can not take solid food enough.

When amino acid solution was given, with maltose mixed, the effect against fermentation in intestine was not so beneficial as had been expected. Rather it would be fruitful if efforts be made to prevent saprophytes from multiplying in the amino acid to be administered.

The cause of diarrhea seems to be attributed, in most part, to the multiplication, fermentation, putrefaction of bacilli. This has been published by Co Tui *et al.*[4]

For calory to be increased addition of maltose has a significance.

In case there is some derangement in absorption like enterocolitis, of course, good results cannot be expected.

The increase rate of weight of sufferers from gastric ulcer is very high, but the increase is not parallel with the disappearance of niches.

Administration through the mouth, though unpalatable, allows a great quantity of the acid compared with that through the skin. Collateral effects are not so marked.[5-6]

Conclusion

I gave to patients of various diseases casein amino acids, defatted soay-bean amino acids and a mixture of the both through their mouth and studied on the fortune of nitrogen, the change of symptoms and other items.

Followings are the conclusions I arrived at.

1. Compared with defatted soya-bean amino acids, casein amino acids are a much more excellent source of nitrogen.

2. Casein amino acids are not only remarkably efficacious to cirrhosis of the liver, but also exercise good influences on other diseases by increasing vigor and weight.

3. Neither casein amino acids nor defatted soya-bean amino acids aggravate the conditions of the patients.

References

1) Co Tui, Wright, Mulholland, Carabba, Barcham & Vinci, Ann. Surg., 1944, **120**, 99.

2) Steinberg, Rev. Gastroent., 1948, **15**, 319.
3) Donald, Edinburgh Med. J., 1951, **58**, 52.
4) Co Tui *et al.*, Gastroenterology, 1945, **5**, 5.
5) Kurota, Kurauchi & Muramatsu, Osaka Ikadaigaku Z, (J. of Osaka Med. Col.,) 1952, **12**, 124.
6) Fuse & Oji, Nippon Rinsho (Jap.), 1949, **7**, 23.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

Biophysikalische Studien über Blutserum-Eiweisse

I. Ueber das gesetzmässige Verhalten der Flockungszahl bei der elektrischen Aufladung des Menschen

Von

Michio Fujii
(藤井 道男)

Aus der II. Medizinischen Universitätsklinik Kobe (Direktor: Prof. Dr. M. Takeda) und dem Medizinisch-chemischen Institut der Toho-Universität Tokyo (Direktor: Prof. Dr. M. Takata)

(Received for publication, June 20, 1953)

I. Einleitung

Die Leistungsfähigkeit der von Maki Takata (1935) eingeführten Flockungszahlreaktion im Serum[1,2,3] ist *in zweifacher Hinsicht* von grosser Bedeutung: *erstens* kann die Reaktion, ähnlich wie die Senkungsgeschwindigkeit der roten Blutkörperchen im Blutplasma, den Grad der Kolloidlabilität der Seren von Patienten zahlenmässig genau angeben (M. Takata[2,3], S. Takata[4], Jezler und Bots[5], Condorelli[6], Kawase[7], Seitz[8], Gohr *et al.*[9,10], Hatashita[11], Sawaki[12] u.a.), und *zweitens* lässt die Flockungszahl (Flz) den Einfluss von Bestrahlungen gesunder Menschen mit harten Röntgenstrahlen, Gamma-Strahlen und Neutronen, also überhaupt durch Strahlen mit einem grossen Durchdringungsvermögen, erkennen und zwar im Sinne einer Steigerung der Flz des kreisenden Blutes (Murasugi)[13]. Gerade die letztgenannte signifikante Eigenschaft der Flockungszahlreaktion ist bedeutungsvoll, macht sie es doch möglich, die Intensität der künstlichen „Vitalionisation" zu bestimmen (vgl. M. Takata[14]) Die *dualistische* Bedeutung der Flockungszahlreaktion für ihre praktische Anwendung ist ursächlich auf Serumproteine verschiedener Zusammensetzung zurückzuführen, die entweder durch normale oder durch pathologische Verhältnisse im Eiweisstoffwechsel bedingt ist. Für eine Verkleinerung der Flz ist hauptsächlich die Zunahme der Globuline, insbesondere γ-Globulin, verantwortlich, für eine erhöhte Flz aber eine andere Komponente des normalen Blutserums, nämlich die „T-Fraktion" (Takata)[14,19], die hier ganz besonders als „Ionenträger" bei der Vitalionisation zur Geltung kommt.

Man wäre wohl noch lange nicht zur Feststellung einer vitalionisier-

baren „Troposphärstrahlung“*, die ursächlich mit solaren Vorgängen zu tun hat, gekommen, wenn nicht erkannt worden wäre, dass die Flockungszahlreaktion im Serum Gesunder zum Nachweis der sonst kaum feststellbaren Vitalionisation gute Dienste leisten kann (M. Takata, Murasugi und S. Takata)[15,16]. Die zusammenfassende Darstellung über die Troposphärstrahlung wurde neulich von M. Takata[17] in einem Wiener Archiv ausführlich berichtet; es würde daher zu weit führen, hier auf diese Strahlung nochmals näher zu kommen**. Ich möchte nur hinzufügen, dass ich mich seit 1949 in Kobe ohne Unterbrechung bis heute noch als einer der Mitarbeiter von Prof. Takata, Tokyo, mit täglichen Reihenmessungen der Flockungszahl beschäftige, um seine kosmobiologische Forschung zu vervollständigen.

Von besonderem Interesse war weiterhin, festzustellen, welchen Einfluss *die elektrische Aufladung des Menschen* auf die Flz ausüben kann. Auf Grund von Orientierungsversuchen an gesunden Personen hat S. Takata (1941)[15] seinerzeit darauf hingewiesen, dass die Flz bei „positiver“ Aufladung proportional der Spannung stets erheblich ansteigt, während sie bei „negativer“ Aufladung mit zunehmender Spannung, wenn auch in schwächerem Ausmasse, so doch immer deutlich abfällt. Unter Leitung von Prof. M. Takata und auf Anregung von Doz. Dr. S. Takata habe ich mich nun mit diesem wichtigen Problem in der Elektrobiologie eingehend beschäftigt und konnte *ein „gesetzmässiges“ Verhalten der Flockungszahl bei der elektrischen Aufladung des Menschen* in eindeutiger Weise bestätigen. Da alle Versuche stets einheitliche Resultate ergaben und jederzeit reproduziert werden können, kann man sagen, dass *eine allgemein gültige Regel für die elektrische Aufladung* jetzt von uns aufgestellt wurde.

---

* Die *Troposphärstrahlung* (Takata) stellt eine elektrisch neutrale, stark vitalionisierende, nahezu masselose Korpuskularstrahlung von enorm starkem Durchdringungsvermögen und Lichtgeschwindigkeit dar, deren Träger höchstwahrscheinlich aus Neutrinos bestehen und durch Zerfallsprozesse der in die Troposphäre eingedrungenen Mesonen bzw. Neutrettos aus Primärstrahlen erzeugt worden sein müssen. Die Primärstrahlen haben ihren Ursprung in der Sonne, die in dem sog. 11 jährigen Zyklus aktiv tätig ist. Die Troposphärstrahlung erleidet bis zu ihrer Ankunft auf der Erde eine immer stärker werdende Absorption durch die Erdatmosphäre [S. Takata (1943)[18]; M. Takata, D. G. Simons, S. Takata, T. Hatashita, K. Kitahara und M. Kagami (1951)]. Sie kann daher derzeit nur durch das von uns benutzte Verfahren — die Messung der Vital-Ionisation des Organismus —, dagegen noch nicht durch eine physikalische Messmethode, erkannt werden.

** Vgl. M. Takata und Murasugi: Bioklim. Beibl. 1941, **8**, 17; H. Berg: Wetter und Krankheiten. H. Bouvier u. Co. Verlag, Bonn 1948, S. 110; M. Curry: Bioklimatik. Medizinisch-Bioklimat. Institut, Riederau am Ammersee 1946, I. Teil, 13. u. 14. Kapitel; II. Teil, 27. Kapitel; K.O. Kiepenheuer: Naturwiss. 1950, **37**, 10, 234.

II. Versuchsteil

Versuchsmethode und -anordnung

Bevor ich nun auf die Versuchsbedingungen beim Anlegen der elektrischen Aufladung eingehe, möchte ich einige grundsätzliche Bemerkungen zur Flockungszahlreaktion und Aufladung vorausschicken. Es gibt eigentlich *drei verschiedene Methoden zur Flockungszahlbestimmung* und zwar 1. die Originalmethode nach Takata-Dohomoto (1936)[1,2,19], in der 0,25 cc. einer 10%igen Sodalösung (aus Na_2CO_3·2 H_2O, E. Merck-Darmstadt) zu 9 Röhrchen mit je 1 cc. Serumverdünnung (1:10) ausgesetzt werden; 2. die Modifikation nach Murasugi (1940)[2,20], in der unter sonst gleicher Bedingung wie oben das mit dem Takata-Reagens zu titrierende Reagensglas (Takata-Röhrchen: 11 mm×8 cm) gleich nach Zusatz adäquater Menge des Reagens unter Vermeidung von Schaumbildung mehrmals stossweise ganz kräftig geschüttelt wird; 3. die Modifikation von Hatashita (1951)[11], in der statt 0.25 cc. einer 10% igen Sodalösung nur 0,11 cc. zu je 9 Röhrchen zugefügt werden. Bei Hatashita's Methode fällt auch ein geringer Teil der Euglobuline mit der Initialflockung der T-Fraktion aus dem normalen Serum aus, was die Ablesung des Reaktionsumschlages bedeutend erleichtert.

Von den genannten Methoden ist das Verfahren von Murasugi[20] besonders gut für die Intensitätsbestimmung der durch Bestrahlung hervorgerufenen Vitalionisation geeignet, da beim Titrieren mit dem Takata-Reagens die als Ionen- bzw. Ladungsträger fungierende „T-Fraktion" aus dem normalen Serum allein in zarter Faserform eher als die Euglobuline, die der Bestimmung des Bestrahlungs- bzw. Aufladungseffektes mehr oder weniger störend im Wege stehen, zur Ausflockung kommt. Aus diesem Grunde halte ich nach meinen reichhaltigen Erfahrungen die Methode von Murasugi zur Messung des Aufladungseffektes für zweckdienlich und habe sie für die folgenden Versuchsreihen bevorzugt. Ueber die Technik von Murasugi's Methode verweise ich aus Platzgründen auf die Originalschrift von M. Takata[2], während ich hier nur darauf aufmerksam mache, dass alle diese 3 Methoden in Takata-positiven, pathologischen Seren immer gleiche Werte der Flz geben, die dem Ausfall der Takata-Reaktion parallel gehen.

Beim elektrischen Aufladungsversuch sind verschiedene Punkte besonders zu beachten, da man sonst zu Irrtümern kommt (vgl. Reissmann, Buettner und Topca[21,22] sowie Sarre[23]). Bei der positiven Aufladung z. B. soll der positive Pol der Batterie stets mit der auf dem Isolierschemel sitzenden Versuchsperson gut verbunden, der negative Pol dagegen gut geerdet sein. Beim Fehlen der Erdung des Gegenpols wird keine Schwankung der Flockungszahl erzielt.

Die Versuchsperson (am besten gesunde, junge Personen!) und der das Blut entnehmende Versuchsleiter sitzen dabei auf einem mit Kupferblech beschlagenen Isolierschemel, der durch Ebonit gegen die Erde isoliert ist. Das Metallblech ist durch ein Drahtkabel an den einen, z. B. positiven Pol der Batterie gebunden, so dass Versuchsleiter und Versuchsperson durch die gleiche

Spannung aufgeladen sind. Der entgegengesetzte, z. B. negative Pol muss dabei durch ein Drahtkabel an die Wasserleitung oder sonstwie gut geerdet werden. Die isolierte Versuchsperson soll die Schuhe ausziehen und mit entblössten, angefeuchteten Füssen auf dem Isolierschemel sitzen. Dann sind die Voraussetzungen für den Aufladungsversuch voll und ganz erfüllt. Zu diesem Zwecke hat M. Takata einen besonderen Apparat konstruiert, bei dem Wechselstrom mit Hilfe einer Gleichrichterröhre gleichgerichtet wird, der dann für die Aufladung dient. Die Spannung des Gleichstromes wird dann durch einen Schiebe-Rheostat nach Belieben einreguliert (Ablesung an einem in den Stromkreis geschalteten Voltmeter). Dieser Apparat stellt einen vollwertigen Ersatz für eine Batterie dar.

Es wurden Aufladungsversuche an gesunden Personen, manchmal aber auch an schweren Kranken, systematisch angestellt. Zu einer Versuchsserie mit positiver und negativer Aufladung wurde, wenn möglich, ein und derselbe Mann gewählt, um die individuell bedingten Unterschiede der Ansprechbarkeit, — z. B. durch Alter, Geschlecht, Gesundheit usw. —, auszuschalten. Zunächst wurde der auf dem Isolierschemel aufsitzenden Versuchsperson Blut (ca. 5 cc. und etwas mehr) entnommen, das den Ausgangswert der Flockungszahl (Flz_o) angibt. Gleichzeitig wurde noch die Takata-Reaktion mit demselben Serum angestellt. Die Blutentnahme muss aber sorgfältig erfolgen, da jede geringe Hämolyse schon das Resultat sehr beeinträchtigt. So soll die Blutentnahme stets mit einer völlig trockenen Spritze und Nadel vorgenommen werden. Die Versuchsperson wird auf +150 Volt, +300 Volt und weiter auf +450 Volt aufgeladen, worauf genau nach 10 Minuten die Blutentnahme erfolgt. Da die Flz, die während der Aufladung eine bestimmte Höhe erreicht, gleich nach dem Ausschalten der Elektrizität sofort wieder zum Ausgangswert zurückgeht, muss das Blut immer während der Aufladung entnommen werden.

Nach Beendigung dieser Reihenversuche wurden die elektrischen Pole umschaltet und die Blutentnahme während der negativen Aufladung wie oben vorgenommen und zwar bei O, −150 Volt, −300 Volt und schliesslich −450 Volt. Zur Prüfung, ob die Versuchsperson richtig aufgeladen ist, verwendet man einen Neon-Tester.

Wählt man den Ausgangswert der Flz als Grundlage zum Vergleich mit den weiteren Proben, so kann der *Aufladungseffekt* (J') einfach nach der Formel: $J' = Flz_x - Flz_o$ (+J') für die positive und $J' = Flz_o - Flz_x$ (−J') für die negative Aufladung berechnet werden, wobei Flz_o den Ausgangswert, Flz_x den Wert einer bestimmten Spannung bedeutet.

Versuchsergebnisse

Wie schon eingangs erwähnt, steigt die Flockungszahl bei der „*positiven*" Aufladung gesunder Personen (z.B. bis zu +450 Volt) annähernd proportional der Spannung stets erheblich an, während sie bei der „*negativen*" Aufladung (z.B. bis zu −450 Volt) mit zunnehmender Spannung, wenn auch in etwas schwächerem Ausmasse, immer deutlich abfällt.

Es ist beachtenswert, dass der Anstieg der Flz bei der positiven Aufladung viel stärker ist als der Abfall bei der negativen Aufladung. *Zwischen der positiven und negativen Aufladung besteht somit hinsichtlich ihres Beeinflussungsmodus eine „reziproke" Beziehung*, die sich in meisten Fällen im Verhältnis 2,6:1 ausdrücken lässt. Dieser Zahlenwert ist ungefähr dem seinerzeit von S. Takata[15] erzielten Wert (2,7:1) gleich. Uebrigens kann die gekoppelte Kurve der positiven und negativen Aufladung im grossen und ganzen als eine Sigmoid-Kurvenform bezeichnet werden (Abb. 1). Weiterhin wurde festgestellt, dass die Flz-Aenderung dabei gar nichts mit dem Gesamt-Eiweissbild und den einzelnen Eiweissfraktionen im Elektrophoresediagramm (vgl. II. Mitteilung) zu tun hat, sondern lediglich mit einem besonderen Eiweisskörper des Blutserums, der als „Ladungsträger" fungieren muss. Hierfür kommt nach unserer Ansicht die *„T-Fraktion"* in Frage, da man ohne diese das charakteristische Phenomen nie nachweisen kann. Diese Theorie hat sich in unserem nachherigen elektrophoretischen Versuch mit dem aufgeladenen Blutserum als zutreffend erwiesen (vgl. II. Mitteilung).

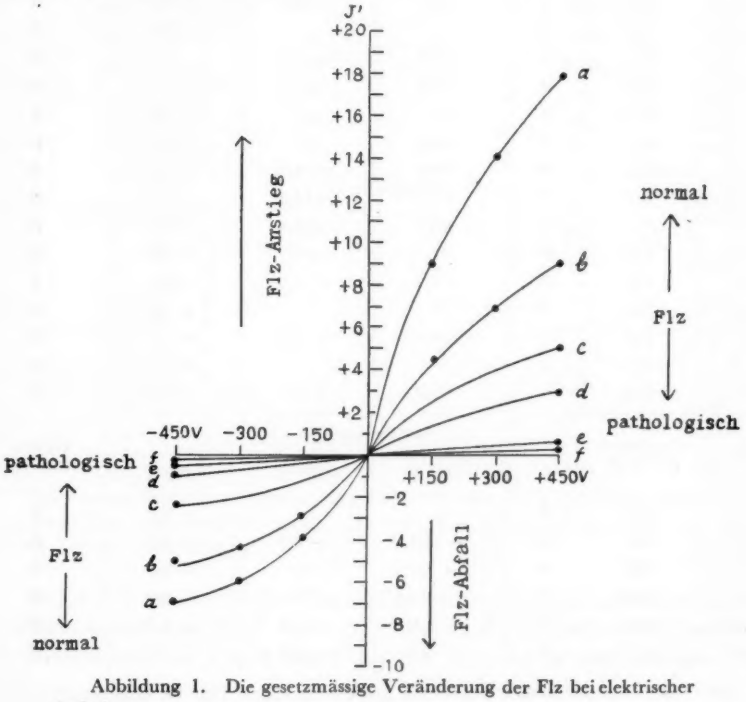


Abbildung 1. Die gesetzmässige Veränderung der Flz bei elektrischer Aufladung.

Diese charakteristische, elektrobiologische Erscheinung ist besonders signifikant bei der Flockungszahlreaktion nach der von Murasugi modifizierten Methode. Vergleicht man diese (Abb. 2, Kurve a) mit den nach der Originalmethode (Abb. 2, Kurve b) und der Modifikation von Hatashita (Abb. 2, Kurve c) erzielten Resultaten, so sind grosse Abweichungen voneinander festzustellen (Tabelle I-III). Bei Unterlassung der Erdung des Gegenpols der Batterie tritt die sonst obligatorisch auftretende Flz-Aenderung nicht mehr zutage (Abb. 2, Kurve d; Tabelle IV).

TABELLE I

Beziehungen des Aufladungseffektes zum Ausgangswert der Flz von verschiedenen Versuchspersonen (nach Murasugi's Methode)

Nr.	Ausgangs-Flz-Wert	Aufladungseffekt (J')				Takata-Reaktion
		0	+150 V.	+300 V.	+450 V.	
1	89,0	0	+9,0	+14.0	+17,0	Ø
2	89,0	0	–	–	+10,0	Ø
3	86,0	0	+8,5	+11,5	–	Ø
4	86,0	0	+8,5	+11,0	–	Ø
5	85,5	0	–	–	+ 9,5	Ø
6	82,5	0	+4,0	–	+ 8,5	Ø
7	75,5	0	+9,0	+14,0	+17,5	Ø
8	74,0	0	+8,5	+13,0	+16,5	Ø
9	73,5	0	+9,0	+14,0	+16,0	Ø
10	73,0	0	–	–	+ 9,0	Ø
11	71,0	0	+8,5	+13,0	+16,5	Ø
12	71,0	0	–	–	+ 8,0	Ø
13	70,5	0	+4,0	+ 7,0	+ 8,5	Ø
14	70,5	0	–	–	+ 8,5	Ø
15	65,0	0	+9,0	+14,0	+18,0	Ø
Nr.	Ausgangs-Flz-Wert	Aufladungseffekt (J').				Takata-Reaktion
		0	−150 V.	−300 V.	−450 V.	
16	61,0	0	–	–	− 3,0	Ø
17	78,0	0	− 5,0	− 6,0	− 6,5	Ø
18	78,0	0	− 5,0	–	− 6,5	Ø
19	85,5	0	–	–	− 6,0	Ø
20	91,0	0	− 3,5	− 5,5	− 6,5	Ø
21	91,0	0	− 4,0	− 6,0	− 7,0	Ø

NB.: Die Untersuchungen wurden im Laufe vom August 1951 bis Oktober 1952 durchgeführt.

Tabelle II

Beziehungen des Aufladungseffektes zum Ausgangswert der Flz von verschiedenen Versuchspersonen (nach Takata-Dohmoto's Originalmethode)

Nr.	Ausgangs-Flz-Wert	Aufladungseffekt (J')				Takata-Reaktion
		0	+150 V.	+300 V.	+450 V.	
1	90,0	0	—	—	+7,0	ø
2	89,0	0	+4,0	+7,0	+8,5	ø
3	87,5	0	+4,0	+7,0	+8,5	ø
4	87,0	0	+4,0	+7,0	+8,5	ø
5	83,5	0	—	—	+5,0	ø
6	82,5	0	+3,5	+6,5	+8,0	ø
7	82,0	0	—	—	+6,0	ø
8	76,0	0	—	—	+7,0	ø
9	74,0	0	—	—	+6,0	ø
10*	64,0	0	—	—	+5,0	ø
11	63,0	0	—	—	+2,5	ø
12**	53,0	0	—	—	+3,0	ø
13	53,0	0	—	—	+2,5	ø
14***	31,0	0	—	—	+0,5	++(4)
15	29,5	0	—	—	+0,5	++(4)
16****	18,0	0	—	—	0	+++(6)
Nr.	**Ausgangs-Flz-Wert**	**Aufladungseffekt (J')**				**Takata-Reaktion**
		0	−150 V.	−300 V.	−450 V.	
17****	18,0	0	—	—	0	+++(6)
18***	31,0	0	—	—	0	++(4)
19**	53,0	0	—	—	−1,0	ø
20	63,0	0	—	—	−1,0	ø
21*	64,0	0	—	—	−2,5	ø
22	93,0	0	−3,0	−5,0	−5,5	ø
23	93,0	0	−4,0	−5,0	—	ø
24	109,0	0	−2,0	−3,0	—	ø

* Die Sternen bedeuten ein und dieselbe Versuchsperson, bei der die Aufladungsversuche am gleichen Tage vorgenommen wurden. Die Versuche wurden auch im Laufe vom August 1951 bis Oktober 1952 gemacht.

Die durch methodische Verschiedenheiten bedingte Abweichung der Resultate hängt wesentlich davon ab, ob sich die störenden Faktoren,d.h. die Euglobuline, an der Ausflockung der T-Fraktion mitbeteiligen oder nicht.

Es fragt sich nun, ob auch *die Flz der pathologischen Seren*, in denen die

TABELLE III

Beziehungen des Aufladungseffektes zum Ausgangswert der Flz von verschiedenen Versuchspersonen (nach Hatashita's Methode)

Nr.	Ausgangs-Flz-Wert	Aufladungseffekt (J')				Takata-Reaktion
		0	+150 V.	+300 V.	+450 V.	
1	75,5	0	+3,0	+5,0	+5,5	ø
2	72,5	0	+2,5	+4,0	+4,5	ø
3	68,5	0	+2,5	+4,0	+4,5	ø
Nr.	Ausgangs-Flz-Wert	Aufladungseffekt (J')				Takata-Reaktion
		0	−150 V.	−300 V.	−450 V.	
4	84,0	0	−5,0	−	−6,5	ø

TABELLE IV

Beziehungen des positiven Aufladungseffektes ohne Erdanschluss des Gegenpols der Batterie zum Flz-Wert von gesunden Personen (nach Takata-Dohmoto's Originalmethode)

Nr.	Ausgangs-Flz-Wert	Aufladungseffekt (J')				Takata-Reaktion
		0	+150 V.	+300 V.	+450 V.	
1	94,5	0	0	0	0	ø
2	93,0	0	0	0	0	ø
3	89,0	0	0	0	0	ø
4	88,0	0	0	0	0	ø
5	77,0	0	0	0	0	ø

Euglobuline bzw. γ-Globuline stark vermehrt sind, durch die elektrische Aufladung ähnlich wie die der normalen Sera ebenso stark beeinflusst wird. Hierzu habe ich mehrere Patienten, unter denen sich solche befanden, die eine geringere Flz und dementsprechend eine positive Takata-Reaktion aufweisen, als Versuchspersonen herangezogen. Es hat sich dabei herausgestellt, dass die Flz dieser Krankensera durch elektrische Aufladung nur schwer oder kaum beeinflusst wird, und zwar *je kleiner die Flz ist, desto geringer ist der Aufladungseffekt. Fällt die Takata-Reaktion im Serum stark positiv aus, so bleibt die Flz-Veränderung, selbst bei starker Aufladung wie z.B. auf* +450 *oder* −450 *Volt, fast vollständig aus* (Abb. 1 und Tabelle II). Ausserdem haben wir elektrophoretisch festgestellt, dass in solchen Fällen γ-Globuline mengenmässig besonders stark vermehrt waren. Daraus geht hervor, dass die pathologisch zugenommenen γ-Globuline die sonst auftretende Steigerung bzw. Absenkung der Flockungszahl bei der Aufladung vollständig unterdrücken. Mit anderen Worten: *Je stärker sich*

TABELLE V

Beziehungen des Aufladungseffektes in vitro zum Flz-Wert (nach Murasugi's Methode)

Nr.	Ausgangs-Flz-Wert	Aufladungseffekt (J')				Takata-Reaktion
		0	+150 V.	+300 V.	+450 V.	
1*	95,5	0	–	–	+3,5	∅
2	93,0	0	–	–	+4,5	∅
3	89,5	0	+9,5	+9,5	+9,5	∅
4	87,5	0	–	–	+8,0	∅
5	87,5	0	–	--	+7,0	∅
6	86,0	0	–	–	+5,0	∅
7**	84,0	0	–	–	+3,0	∅
8***	82,0	0	+5,0	+5,0	+5,0	∅
9	79,5	0	–	–	+2,5	∅
10	78,5	0	+5,0	+5,0	+5,0	∅
11	72,0	0	+4,0	+4,0	+4,0	∅
12****	69,0	0	–	–	+3,5	∅
13*****	66,0	0	--	–	+3,5	∅
14******	64,0	0	–	–	+3,5	∅
15*******	62,0	0	–	–	+4,5	∅
16*******	62,0	0	--	–	+4,0	∅
17	53,0	0	–	–	+1,0	∅
18********	38,5	0	–	–	+0,5	+(3)
19********	29,5	0	–	–	0	++(4)
20*********	16,5	0	–	–	0	+++(7)
21*********	11,0	0	–	–	0	+++(8)

Nr.	Ausgangs-Flz-Wert	Aufladungseffekt (J')				Takata-Reaktion
		0	−150 V.	−300 V.	−450 V.	
22*********	11,0	0	–	–	0	+++(8)
23*********	16,5	0	–	–	0	+++(7)
24*********	29,5	0	–	–	0	++(4)
25*********	38,5	0	–	–	−0,5	+(3)
26********	62,0	0	–	–	−2,5	∅
27********	62,0	0	–	–	−3,0	∅
28*********	64,0	0	–	–	−1,0	∅
29*********	66,0	0	–	–	−2,0	∅
30*******	69,0	0	–	–	−1,5	∅
31	72,0	0	−2,0	−2,0	–	∅
32***	82,0	0	–	–	−2,0	∅
33***	84,0	0	–	–	−2,0	∅
34*	95,5	0	–	–	−1,5	∅

* Die Sternen bedeuten ein und dasselbe Blutserum. Die Versuche wurden im Jahre 1952 durchgeführt.

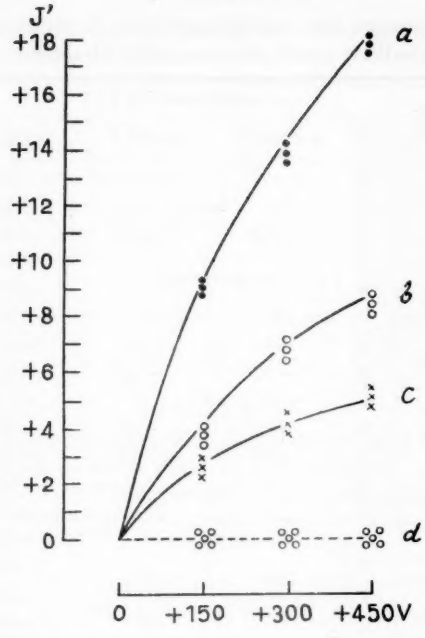


Abbildung 2. Verhalten der Flz bei der positiven Aufladung von ein und derselben Versuchsperson (Fujii ♂ 30 j., gesund). Die Flz wurde bestimmt nach der Methode von Murasugi (Kurve a), nach der Original-Methode (Kurve b) und nach der Modifikation von Hatashita (Kurve c). Die Flz wurde noch nach der Originalmethode ohne Erdanschluss des Gegenpols der Batterie bestimmt (Kurve d).

die γ-Globuline bzw. Euglobuline vermehren, desto geringer ist der Aufladungseffekt und umgekehrt. Dies ist auch der Grund, weshalb diese Patienten für den Aufladungsversuch ungeeignet sind; weiter geht daraus hervor, dass *den Euglobulinen ganz und gar keine Funktion als Ladungsträger zukommt.*

Es wurden weiter noch *Aufladungsversuche in vitro* vorgenommen, um festzustellen, ob auch im Vitro-Versuch mit dem Serum ein ähnlicher Effekt wie in vivo auftreten kann. Die Versuche ergaben, dass sich die Flz des normalen Serums, dem nur kurzdauernd (durchschnittlich 2 Min.) eine bestimmte Spannung angelegt war, zwar wie üblich verändert, jedoch im allgemeinen nur geringgradig. Die Flz ist dabei unabhängig von der Spannung, und hält sich stets auf derselben Höhe (Tabelle V). Hier wurde wieder festgestellt, dass in Takata-positiven pathologischen Seren der Aufladungseffekt vollständig ausbleibt.

Warum die Flockungszahl der Seren von gesunden Personen in vivo durch die Aufladung z.B. auf +450 Volt bei der einen um J'+18 und bei der anderen nur um J'+8 anstieg, könnte man wohl mit einer individuell differenten Ansprechbarkeit des lebenden Organismus auf statische Elektrizität erklären.

Wie schon erwähnt, hängt der Aufladungseffekt offenbar von der Grösse der Ausgangs-Flockungszahl ab. Solange es sich um normale Seren handelt, schien der Aufladungseffekt nicht immer so abzunehmen, dass sich die Flz, nach ihrer Zahlenfolge angeordnet, stufenweise von oben nach unten (bei positiver Aufladung) oder von unten nach oben (bei negativer Aufladung) gleichmässig verkleinert (Tabelle I-III). Dieser scheinbare Widerspruch könnte dadurch erklärt werden, dass die Flz zur Zeit unserer Experimente selbst bei ein und derselben Versuchsperson immer noch einer starken *Tagesschwankung* unterworfen war und infolgedessen von Tag zu Tag variierte Werte zeigte (sog. „Flockungszahl-Störungen"). Die normale Flz in den Flz-Störungszeiten beträgt 60–100, manchmal sogar noch mehr. Werte von 50 bis 60 sind subnormal und unter 50, worauf auch Gohr schon hinwies, zweifellos pathologisch.

III. Besprechung

Schon von früher her kennen wir *wichtige Aufgaben des Bluteiweisses*, die offenbar mit den physikalisch-chemischen Eigenschaften der Plasmaproteine zu tun haben. So z. B. die *Vehikelfunktion* zum Abtransport verschiedener Stoffe, *der kolloidosmotische Druck* zur Balanzierung des hydrostatischen Druckes in den Kapillaren, die *Pufferwirkung* zur Aufrechterhaltung einer konstanten Blutreaktion usw. Eine weitere physiologische Funktion der Plasmaproteine von ebenfalls physikalisch-chemischer Art besteht in ihrem Beitrag zur *Adsorption der während der Bestrahlung in vivo entstandenen Ionen*. Bei der Vital-Ionisation tritt die „T-Fraktion" des Blutserums als Ionenträger in Funktion. Betrachten wir nun die Abhängigkeit der Intensität der Vital-Ionisation (J') von der Bestrahlungszeit (t), so finden wir, dass die Bestrahlungszeits-Intensitätskurve eine Adsorptionsisotherme darstellt. Trägt man die Logarithmen der Bestrahlungszeit auf der Abszisse, und der Ionisationsintensität auf der Ordinate auf, so erhält man eine gerade Linie. Auch hier könnte *die ausschlaggebende Bedeutung der T-Fraktion auf die Adsorption der in vivo entstandenen Ionen* nachgewiesen werden.

Bedeutungsvoll ist, dass *die durch harte Röntgenstrahlen ausgelöste Vital-Ionisation*, ähnlich wie die Luftionisation, einem allgemeinen „Gesetz" folgt (Murasugi)[13,14]. So ist die Stärke der Vital-Ionisation (J') proportional dem Quadrat der Röhrenspannung, umgekehrt proportional dem Quadrat des Abstandes von der Strahlenquelle und direkt proportion-

al der Feldgrösse. Ferner wurde noch eine Wellenlängenabhängigkeit bei der Vital-Ionisation festgestellt, wonach nur die harte, d.h. kurzwellige Röntgenstrahlung für die Vital-Ionisation verantwortlich ist.

Die *positive Aufladung des Menschen* zeigt nun mit auffälliger Steigerung der Flz-Kurve eine der Vital-Ionisation verwandte Erscheinung. Diese Befunde lassen die Deutung zu, dass sich die Ionen oder Ladungen an bestimmten Fraktionen der Serumproteine selektiv anhäufen müssen. In der Tat wurde von mir elektrophoretisch festgestellt, dass der Gipfel der γ-Globuline sowohl des normalen (d.h. des Takata-negativen) wie auch des pathologischen (d.h. des Takata-positiven) Blutserums durch eine „positive", nicht dagegen durch eine negative Aufladung der Personen in zwei Zacken (γ_1 und γ_2) aufgespalten wird (vg. II. Mitteilung). Diese überaus wichtige Feststellung führt uns zu dem Schluss, dass γ_2-Globulin zweifellos mit der „T-Fraktion" (Takata) identisch sein müsse, und als „Ladungsträger" bei der elektrischen Aufladung des Menschen fungiert. Diese Annahme ist insofern richtig, als *die T-Fraktion insofern ganz besonders als Ladungsträger in Funktion tritt*, als ohne sie ein Aufladungseffekt nicht mehr nachweisbar ist. Auf Einzelheiten werde ich in der nächsten Mitteilung noch zurückkommen.

IV. Zusammenfassung

Es wurden systematische Untersuchungen über das Verhalten der Flockungszahl bei der elektrischen Aufladung des Menschen angestellt und folgende Resultate gewonnen:

1. Bei der elektrischen Aufladung verändert sich die Flz nach einem allgemeinen „*Gesetz*" deutlich, indem positive Aufladung der isolierten Versuchsperson die Flz erhöht, während negative Aufladung sie vermindert. Die Variation der Flz ist bei der positiven Aufladung viel grösser als bei der negativen Aufladung. (Etwa um den Wert +18 bei +450 Volt, um −8,5 bei −450 Volt bei ein und derselben gesunden Person).

2. Der Aufladungseffekt bleibt völlig aus, wenn der Gegenpol der Batterie nicht geerdet ist. Als wichtigste Vorbedingungen für einen erfolgreichen Aufladungseffekt gilt also vor allem der Erdanschluss des Gegenpols der Batterie!

3. Der Aufladungseffekt ist, ähnlich wie der Bestrahlungseffekt, nur während der Aufladung der Personen nachweisbar und nicht mehr nach dem Ausschalten der Elektrizitätsbelastung, weil die Flz immer nur den Wert zeigt, den das Blut im Augenblick der Blutentnahme hatte. Gleich nach Beendigung der Aufladung kehrt der erhöhte oder verminderte Flz-Wert sofort wieder zum Ausgangswert zurück.

4. Die nach der Modifikation von Murasugi bestimmte Flz nimmt bei der positiven Aufladung von ein und derselben Versuchsperson

um das Doppelte gegenüber dem nach der Originalmethode von Takata-Dohmoto ermittelten Flz-Wert und um das Dreifache im Vergleich zur Modifikation von Hatashita zu. (Etwa um den Wert +18 bei +450 Volt nach der Modifikation von Murasugi, um +8 bei +450 Volt nach der Originalmethode und um +5 bei +450 Volt nach der Modifikation von Hatashita).

5. Der Aufladungseffekt ist bei Patienten, bei denen die Flz-Werte infolge von Krankheitsprozessen wesentlich kleiner sind, weniger deutlich ausgeprägt. Er bleibt dann fast vollständig aus, wenn die Takata-Reaktion im Serum stark positiv ausfällt, und dementsprechend die Flz von vornherein kleiner als 30 ist.

6. Zwischen dem Ausgangs-Flockungszahlwert und dem Aufladungseffekt besteht eine enge Beziehung: Je höher die Ausgangs-Flz ist, desto grösser ist der Aufladungseffekt, doch ist die Grösse des Aufladungseffektes individuell ziemlich variabel.

7. Es wurde elektrophoretisch festgestellt, dass der „T-Fraktion", einer neuen Komponente der Serumproteine, eine Funktion als Ladungsträger zukommt, dass aber γ-Globuline bzw. Euglobuline damit gar nichts zu tun haben.

Literatur

1) Takata, M. u. Dohmoto, Tohoku J. Exp. Med., 1936, **28**, 522.
2) Takata, M., Helv. Med. Acta (Ser. A), 1950, **17**, 254.
3) Takata, M., Die Medizinische, 1952, 617.
4) Takata, M. u. Takata, S., Sei-kagaku (Jap.), 1952, **24**, 1.
5) Jezler u. Bots, Klin. Wschr., 1938, **33**, 1140.
6) Condorelli, Klin. Wschr., 1939, **39**, 1296.
7) Kawase, Shokakibyo-gaku (Jap.), 1939, **4**, 199.
8) Seitz, Tohoku J. Exp. Med., 1938, **33**, 230.
9) Gohr, Falkenbach u. Fincke, Zschr. klin. Med., 1951, **148**, 518.
10) Gohr, Falkenbach u. Langenberg, Zschr. gesamt. inn. Med., 1951, **6**, 251.
11) Hatashita, noch nicht publiziert.
12) Sawaki, noch nicht publiziert.
13) Murasugi, Tohoku Igaku Zasshi (Jap.), 1944, **34**, 213; **34**, 314.
14) Takata, M., Radiologia Clinica, 1951, **20**, 21.
15) Takata, M., Murasugi u. Takata, S., Kessei Gaku Menekigaku Z. (Acta Serol. et Immun.), 1941, **2**, 165.
16) Takata, M. u. Murasugi, Bioklimat. Beibl., 1941, **8**, 17.
17) Takta, M., Arch. Meteorol., Geophysik u. Bioklimat. (Ser. B), 1951, **2**, 486.
18) Takata, S., Tohoku J. Exp. Med., 1949, **50**, 87.
19) Bayer-Labor-Blatt, 1952, 42.
20) Murasugi, Kessei Gaku Menekigaku Z. (Acta Serol. et Immun.), 1940, **1**, 385.
21) Buettner, Bull. American Meteorol. Soc., 1951, **32**, 183.
22) Reissmann, Buettner und Topka, zit. nach Buettner (21)).
23) Sarre, Medizin-meteorol. Hefte, 1951, **5**, 25.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

Biophysikalische Studien über Blutserum-Eiweisse

II. Ueber den Einfluss elektrischer Aufladung des Menschen auf einzelne Serum-Eiweissfraktionen im Elektrophoresediagramm*

Von

Michio Fujii
(藤井 通男)

Aus der II. Medizinischen Universitätsklinik Kobe (Direktor: Prof. Dr. M. Takeda) und dem Medizinisch-chemischen Institut der Toho-Universität Tokyo (Direktor: Prof. Dr. M. Takata)

(Received for publication, June 20, 1953)

I. Einleitung

Die in der ersten Mitteilung[1] geschilderten Forschungen über das gesetzmässige Verhalten der Flockungszahl (Flz) bei der elektrischen Aufladung des Menschen liessen die wichtige Frage aufkommen, ob die „T-Fraktion" (Takata)[2] als Ladungsträger für die Flz-Veränderungen verantwortlich sei. Zur Lösung dieser Frage habe ich das Verhalten einzelner Komponenten der Serum-Eiweisse, besonders aber der T-Fraktion, bei der elektrischen Aufladung elektrophoretisch untersucht.

II. T-Fraktion, ihre physiologische und pathologische Bedeutung

Die „T-Fraktion" stellt eine neue Komponente des menschlichen Serum-Eiweisses dar, welche von Maki Takata (1935) mit Hilfe der von ihm inaugurierten Flockungszahlreaktion im normalen Serum[2] gefunden wurde, wo sie in nur geringer Menge vorhanden ist. Sie ist gegenüber dem Takata-Reagens ausserordentlich empfindlich und fällt beim Titrieren mit dem Takata-Reagens aus soda-alkalisierten normalen Seren (pH 9,6) als Initialflockung in zarter Faserform allererst aus. Bei ihrer Ausflockung aus Takata-positiven pathologischen Seren ist sie in den allzu reichlichen Globularproteinen, d.h. Euglobulinen, eingeschlossen, so dass sie in der Takataflockung kaum zu sehen ist. S. Takata (1940)[3] ist es gelungen, durch „*Harnstoff-Differenzierung*" nachzuweisen, wie reichlich die T-Flockungen in der Takataflockung eingebettet enthalten sind.

* Die vorliegende Arbeit wurde auch unter Leitung von Prof. Maki Takata durchgeführt.

Durch die Harnstoff-Differenzierung wird *die Trennung der T-Fraktion von den Euglobulinen* leicht möglich, indem die letzteren vorerst vollständig in Lösung gehen und die noch zurückbleibenden T-Flockungen dann optisch sichtbar werden. Daraus geht hervor, dass die *Takataflockung* mindestens *aus zwei Komponenten*, und zwar aus Gemischen der pathologisch vermehrten Euglobulinen (Sphäroproteine) und der ebenfalls zugenommenen T-Fraktion (eine Art der Linearproteine), bestehen. Die von *Wuhrmann* und *Leuthardt* (1938)[4] durch die Plasmalöslichkeitskurven (fraktionierte Salzfällung) aufgefundenen, asymmetrischen „*Takata*-Proteine", welche unter krankhaften Verhältnissen im Fibrinogen- und Euglobulinbereich ausfallen und elektrophoretisch mit den γ-Globulinen wandern, dürften also, wenn wir den Begriff der Takata-Proteine von Wuhrmann[5] als ‹Proteine, die bei der fraktionierten Aussalzung nur im Fibrinogenbereich ausfallen, jedoch nicht zum Fibrinogen gehören›, aufnehmen, höchstwahrscheinlich mit der „*T-Fraktion*" (Takata) identisch sein.

Andererseits haben *Edsall et al.* (1947)[6-7] aus der an Fibrinogen reichen Fraktion I des menschlichen Blutplasmas ein asymmetrisches, ungerinnbares Protein isoliert, das durchschnittlich eine gleiche Länge wie Fibrinogen besitzen soll. Weiter noch wurde von den Autoren festgestellt, dass dieses Protein ein „*dimer*" des Fibrinogen ist, das aber nicht fähig ist, an der Fibrinbildung teilzunehmen (vgl. Pedersen[8]). Die Annahme, dass diese neue Komponente mit der T-Fraktion identisch sein dürfe, muss aus obigem Grunde als sehr wahrscheinlich anzusehen sein.

Der Name *T-Fraktion* (Takata) kann leicht zu Verwechselungen führen, einmal mit der von Deutsch *et al.* (1946)[9] für identisch mit dem γ_1 gehaltenen *T-Komponente* des Pferde-Immunserums oder sogar mit einer von Wallis (1950)[10] „*T*" genannten Serum-Eiweisskomponente mit der Wanderungsgeschwindigkeit des Fibrinogens (vgl. Antweiler[11]), als auch mit dem von Smith und Gerlough (1947)[12] aus dem Pferde-Immunserum isolierten *T-Globulin*, da die Bezeichnungen miteinander ziemlich ähnlich sind. Dass alle diese Begriffe voneinander verschieden sind, dürfte aus später noch zu erörternden Gründen klar werden.

Von Wuhrmann und Wunderly[5] wurde festgestellt, dass die Takata-Proteine bei der Elektrophorese mit den γ-Globulinen, zum Teil wahrscheinlich auch mit den β-Globulinen, wandern. Dass die pathologisch stark vermehrten *γ-Globuline für den positiven Ausfall der Takata-Reaktion unmittelbar verantwortlich* sind, wurde von vielen Autoren (Wuhrmann und Wunderly,[5] Staub,[13] MacLagan und Bunn,[14] Olhagen,[15] Gohr *et al.*,[16,17] Albertsen, Christoffersen und Heintzelmann,[18] Eymer und Zöllner,[19] Lasch und Nowak,[20] S. Takata, Fujii und Otani,[21] Wakamatsu,[22] Nabekura,[23]

Yoshida und Sumitomo,[24] Fujiyama,[25] Nomura,[26,27] Ueda[28] u.a.) genügend bewiesen. Ebenso wurde von M. Takata und Hatashita (1952) durch Anwendung der nach dem Aethanol-Fraktionierungsverfahren von Cohn aus dem normalen menschlichen Blutplasma rein dargestellten γ-Globuline der Nachweis erbracht, dass die γ-Globuline überhaupt gar keine Kolloidschutzwirkung besitzen (Flz 0,5 und Takata-Reaktion extrem stark positiv; Takata-Fuchsinzahl Null)[30]. Die Flockungszahl wird also bei starker Vermehrung der Globuline erheblich kleiner.[31] *Zwischen dem Ausfall der Takata-Reaktion und dem Flz-Wert besteht somit eine enge Korrelation.* Es liegt daher nahe, anzunehmen, dass die γ-Globuline ebenso wie die Takata-Flockung mindestens aus zwei Hauptkomponenten bestehen müssen. Ferner konnten M. Takata und Hatashita,[29,31] S. Takata und Fujii,[30] Yoshida und Sumitomo[24] sowie Takeuchi[32] zeigen, dass die Lipoid-tragenden Globuline, vor allem die β-Globuline, sowohl in vivo wie auch in vitro die Takata-Reaktion und die Flockungszahlreaktion hemmen.

Durch Elektrophorese wurde klar gestellt, dass sich die γ-Globuline tatsächlich aus zwei Unterfraktionen, γ_1 und γ_2, zusammensetzen, des weiteren, dass das γ-Plasmocytom, bei dem entweder γ_1- oder γ_2-Globulin überwiegend erheblich zunimmt, meist die stärkste Zunahme der im Fibrinogen-Bereich fällbaren Takata-Proteine zeigt (Wuhrmann und Wunderly)[5]. Eine scharfe Trennung dieser beiden Unterfraktionen ist aber bei sonstigen anderen Erkrankungen sehr schwer. Es ist nun Wiedemann[33] gelungen, elektrophoretisch festzustellen, dass auch das normale menschliche Serum eine geringe Menge von γ_2-Globulin enthält, das aber nur durch Extrapolation erkennbar ist. Es kam jetzt darauf an, endgültig zu entscheiden, welcher Anteil von diesen beiden Unterfraktionen mit der T-Fraktion identisch ist.

Uns ist es endlich gelungen, durch eine „positive" Aufladung des Menschen den elektrophoretisch nachweisbaren Gipfel des γ-Gradienten sowohl vom normalen (d.h. Takata-negativen) wie auch vom pathologischen (d.h. Takata-positiven) Blutserum, wenn auch manchmal nicht so prägnant, doch noch in zwei Zacken aufzutrennen. Diese allerwichtigste Feststellung in der Elektrophorese führt uns zu dem Schluss, dass *γ_2-Globulin zweifellos mit der T-Fraktion identisch* sein dürfte, und dass es sogar, wie wir seit langem annehmen, als *„Ladungsträger"* bei der elektrischen Aufladung des Menschen fungiert.

III. Die Trennung der γ-Globuline in 2 Unterfraktionen durch elektrische Aufladung

Wie schon erwähnt,[1] steigt die Flockungszahl bei der „positiven" Aufladung gesunder Personen (z.B. bis zu +450 Volt) proportional der Spannung stets erheblich an, während sie bei der „negativen" Aufladung

(z.B. bis zu −450 Volt) mit zunehmender Spannung immer deutlich abfällt. Die „gesetzmässige" Veränderung der Flz bei der elektrischen Aufladung würde uns gestatten, Rückschlüsse auf die Existenz eines als „Ladungsträger" fungierenden Bluteiweisskörpers besonderer Art zu ziehen. Hierfür ist nach unserer Ansicht Voraussetzung die T-Fraktion, da ohne diese das signifikante Phenomen nie nachzuweisen ist.

Theoretisch wird angenommen, dass der Anstieg der Flz bei der positiven Aufladung durch eine Anhäufung positiver Ionen an der Oberfläche der Teilchen der T-Fraktion herbeigeführt wird, während der Abfall der Flz bei negativer Aufladung durch eine Vermehrung negativer Ionen in den Molekülen der T-Fraktion zustande kommt. Sollte diese Hypothese sich als richtig erweisen, so müsste eine entsprechende Veränderung im Elektrophoresediagramm der elektrisch aufgeladenen Seren nachzuweisen sein.

Um dies elektrophoretisch festzustellen, wurden Untersuchungen vorgenommen, die eindeutige Resultate ergaben. Hierfür wurden Versuchspersonen 20 Minuten lang entweder positiv oder negativ jeweils auf 450 Volt aufgeladen, und die Elektrophoresebilder vor, während und nach der Aufladung beobachtet.

Wie Tabelle I zeigt, nahm der *Albumin-Gradient* in fast sämtlichen Versuchsfällen mehr oder weniger deutlich ab und der *γ-Gradient* dagegen ausnahmslos zu, ganz gleich, ob es sich um eine positive oder um eine negative Aufladung handelte. Man sieht also hier keine spezifischen Befunde. *Ganz charakteristisch und eindrucksvoll aber war es, dass eine Auftrennung der γ-Globuline in ihre 2 Unterfraktionen, und zwar in eine γ_1- und γ_2-Fracktion, bei „positiver", nicht dagegen bei negativer Aufladung gelang* (Abb. 1).

Es wurde schon von Wuhrmann, Wunderly und de Nicola[34,35] darauf hingewiesen, dass bei Vermehrung von sogenannten „heterogenen" γ-Globulinen das elektrophoretische Bild ganz charakteristisch eine breitbasige, relativ wenig hohe und abgerundete Höckerbildung an Stelle einer eigentlich schmalen, hohen und spitzen γ-Globulinzacke erkennen lässt. Die in der breitbasigen γ-Zacke vorhandenen Dysproteine entsprechen dann wohl den „Takata-Proteinen", die nach Wuhrmann in Plasmalöslichkeitskurven (fraktionierte Salzfällung) im Fällungsbereich des Fibrinogens ausfallen und nicht identisch mit dem Fibrinogen sind (vgl. auch Emmrich[36]). Das von uns erzielte elektrophoretische Bild des positiv aufgeladenen Blutserums zeigt ebenfalls, soweit es sich um die zweite Zacke der γ-Globuline handelt, etwas Aehnliches.

Der von uns nachgewiesene Befund, dass ein Höcker der γ-Globuline durch eine positive Aufladung in zwei Zacken aufgespalten wurde, wäre jetzt wie folgt zu erklären: Infolge der Anhäufung positiver Ionen hinkt dieser Anteil der T-Fraktion, der sonst mit gleicher oder beinahe gleicher Geschwindigkeit

TABELLE I

Beziehungen der prozentualen Anteile diverser Serum-Eiweissfraktionen bei der elektrischen Aufladung zu Flz-Werten

Name	Diagnose	Takata-Reaktion	Aufladung	Relative %				A/G-Quot.	Flz.
				Albumin	α-Glob.	β-Glob.	γ-Glob.		
Fujii ♂ 30 j.	gesund*	∅	in vitro						
			0	53,9	9,0	13,0	24,1	1,17	84,0
			−450 V.	41,7	8,3	11,4	38,6	0,72	82,0
			+450 V.	51,8	7,5	11,7	29,0	1,07	87,0
Otani ♂ 26 j.	gesund*	∅	in vivo						
			0	53,5	4,7	11,5	30,3	1,15	78,0
			−300 V.	51,1	7,2	10,8	30,9	1,04	72,0
			−450 V.	55,3	3,1	10,1	31,5	1,24	71,5
Sakagami ♀ 25 j.	Raynaud-sche Kht	∅	in vivo						
			0	48,4	9,4	10,6	31,6	0,93	53,0
			−450 V.	35,3	11,8	11,8	41,1	0,55	52,0
			+450 V.	35,0	5,5	8,5	51,0	0,54	56,0
Tawara ♂ 35 j.	Lungen-tbc	++	in vivo						
			0	51,6	4,5	15,7	28,2	1,07	31,0
			−450 V.	37,9	4,1	18,5	39,5	0,61	31,0
			+450 V.	48,8	6,0	9,1	36,1	0,95	31,0
Makimoto ♂ 34 j.	Lungen-tbc	###	in vivo						
			0	32,5	12,1	13,0	42,4	0,48	18,0
			−450 V.	24,5	7,3	14,6	53,6	0,32	18,0
			+450 V.	26,1	8,8	12,1	53,0	0,35	18,0

* Die elektrophoretische Auswertung wurde mittels der Tiselius-Mikroapparatur (Fa. Hitachi, Tokyo) gemacht. Relativ grosse Abweichungen der angegebenen Normalwerte von den internationalen Standard-Normalwerten nach Dole[37] (zit. nach Knedel[38]) mögen wahrscheinlich durch Verwendung der Mikroapparatur bedingt sein, die bekanntlich keine befriedigende Abhilfe schafft.

wie die γ-Globuline mit sämtlichen Eiweisskomponenten zur Anode wandert, nunmehr nach. γ_1-Globulin, das an und für sich kein Ladungsträger ist, wandert dabei mit der üblichen Geschwindigkeit, wodurch der Gipfel der γ-Globuline mehr oder weniger deutlich aufgetrennt wird. Bei negativer Aufladung findet dagegen eine starke Anhäufung negativer und Einbusse positiver Ionen an den Teilchen der T-Fraktion ($=\gamma_2$) statt, so dass bei der Wanderung zur Anode die T-Fraktion, die im Eiweiss-Spektrum der Seren neben γ_1 liegt, das vorangehende γ_1 nicht überholen kann. Eine Auftrennung der γ-Globuline kann deshalb nicht erzielt werden.

Auf Grund obiger Feststellung sind wir zu dem Schluss gekommen, dass *die „T-Fraktion" bei der elektrischen Aufladung des Menschen tatsächlich als „Ladungsträger" in Funktion tritt und höchstwahrscheinlich mit dem γ_2-Globulin identisch ist.* Diese Feststellung lässt den Rückschluss zu, dass auch die elektrophoretisch nachweisbaren *γ-Globuline mindestens aus zwei chemisch verschiedenen Bestandteilen, d.h. Euglobulin und T-Fraktion, bestehen, was wiederum*

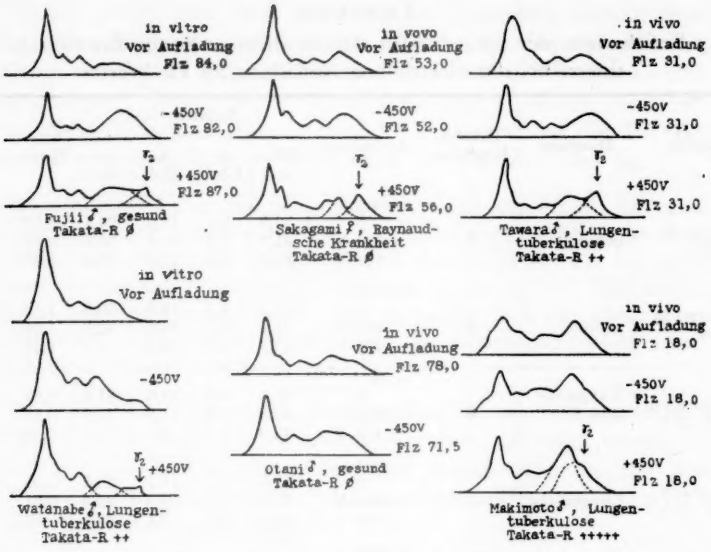


Abbildung 1. Auftrennung der γ-Globuline in eine γ_1- und γ_2-Komponente durch positive Aufladung.

der Treunbarkeit der Takata-Flockung durch die Harnstoff-Differenzierung[2] *entspricht. Die γ-Globuline sind demnach immer mehr oder weniger „heterogene" Gemische.*

Es sei hier noch darauf hingewiesen, dass die Zustandsänderung der „patterns" schon binnen 30 Minuten nach beendeter Aufladung des Menschen wieder zum Ausgangsbilde vor der Aufladung zurückkehrt (Tabelle II). Das Zustandekommen der elektrophoretischen Auftrennung der γ-Globuline in zwei Zacken gilt nicht nur für Patienten mit positiver, sondern auch mit negativer Takata-Reaktion. Auch bei der positiven Aufladung des Blutserums (in vitro) kann eine elektrophoretische Auftrennung der γ-Globuline eintreten, nicht dagegen bei der negativen Aufladung (Abb. 1).

Tabelle I lässt erkennen, dass die Flz der Seren unter krankhaften Verhältnissen von dem Ausfall der Takata-Reaktion und von der Art der elektrischen Aufladung wesentlich abhängig ist. Es liegen jedoch keine eindeutig fassbaren Beziehungen der Anteile der Serum-Eiweiss-komponenten zueinander und zu den Flz-Veränderungen bei der Aufladung vor. Allerdings steht der *Albumin-Globulin-Quotient*, der bei der elektrischen Aufladung stets erheblich absinkt (siehe auch Tabellen II und III), offensichtlich in *keiner* ursächlichen Beziehung zur gesetz-

Tabelle II

Prozentuale Anteile diverser Serum-Eiweissfraktionen bei der elektrischen Aufladung des Menschen

Name	Datum	Aufladung	Relative %				A/G-Quot.	Gesamt-Eiweiss
			Albumin	α-Glob.	β-Glob.	γ-Glob.		
Sakagami ♀ 25 j.	16.2.52	vor Aufladung	39,6	11,8	18,6	30,0	0,64	9,1%
		−450 V. (20′)	32,6	7,9	13,9	45,6	0,48	
		+450 V. (20′)	38,0	6,4	10,8	44,8	0,61	
Tawara ♂ 35 j.	21.2.52	vor Aufladung	54,9	3,8	7,3	34,0	1,21	6,9%
		−450 V. (20′)	45,6	6,1	15,9	32,4	0,83	
		+450 V. (20′)	49,5	5,9	10,8	33,8	0,97	
Makimoto ♂ 34 j.	20.2.52	vor Aufladung	30,1	5,5	14,8	49,6	0,43	7,0%
		−450 V. (20′)	27,4	7,3	9,7	55,6	0,37	
		+450 V. (20′)	28,1	6,8	11,5	53,6	0,39	
Kishimoto ♀ 25 j.	25.4.52	vor Aufladung	55,8	6,7	10,4	27,1	1,26	8,0%
		+450 V. (20′)	50,4	5,2	7,5	36,9	1,01	
		10 Min. nach Beendung	47,3	6,2	11,1	35,4	0,89	
		30 Min. nach Beendung	55,0	7,9	14,3	22,8	1,22	
Kishimoto ♀ 25 j.	25.5.52	vor Aufladung	66,9	7,5	11,5	14,1	2,02	8,2%
		+450 V. (20′)	46,4	5,7	10,8	37,1	0,86	
		10 Min. nach Beendung	44,8	10,3	16,3	28,6	0,81	
		30 Min. nach Beendung	49,3	5,0	11,4	34,3	0,97	
Fujii ♂ 30 j.	28.4.52	vor Aufladung	55,2	6,9	10,6	27,3	1,23	7,4%
		+450 V. (20′)	51,7	5,2	12,2	30,9	1,07	
		10 Min. nach Beendung	52,9	6,2	13,6	27,3	1,12	
		30 Min. nach Beendung	56,1	5,4	13,5	25,0	1,28	
Fujii ♀ 28 j.	3.7.52	vor Aufladung	51,5	7,4	10,9	30,2	1,06	7,2%
		+450 V. (20′)	48,9	8,5	8,5	34,1	0,96	
		10 Min. nach Beendung	45,5	5,3	10,6	38,6	0,83	
		30 Min. nach Beendung	48,3	8,1	10,1	33,5	0,93	
Igaki ♀ 24 j.	11.9.52	vor Aufladung	55,2	7,2	9,8	27,8	1,23	7,2%
		+450 V. (20′)	43,4	7,9	11,3	37,4	0,77	
		10 Min. nach Beendung	46,6	9,1	11,8	32,5	0,87	
		30 Min. nach Beendung	49,3	7,0	9,2	34,5	0,97	
Igaki ♀ 24 j.	8.10.52	vor Aufladung	56,2	8,1	12,3	23,4	1,28	7,3%
		+450 V. (20′)	51,2	5,6	11,4	31,8	1,05	
		10 Min. nach Beendung	52,9	7,2	12,9	27,0	1,12	
		30 Min. nach Beendung	59,3	6,0	10,2	24,5	1,45	
Fujii ♂ 30 j.	3.6.52	vor Aufladung	62,3	7,0	11,5	19,2	1,65	7,2%
		−450 V. (20′)	44,7	7,8	11,9	35,6	0,81	
		10 Min. nach Beendung	54,5	9,5	12,7	23,3	1,19	
		30 Min. nach Beendung	60,4	5,8	11,7	22,1	1,52	
Takeuchi ♀ 34 j.	1.10.52	vor Aufladung	55,1	8,0	6,4	30,5	1,23	7,4%
		−450 V. (20′)	38,5	10,6	10,8	40,1	0,63	
		10 Min. nach Beendung	42,6	11,4	11,7	35,3	0,73	
		30 Min. nach Beendung	51,4	7,4	8,5	32,7	1,06	

TABELLE III

Prozentuale Anteile der Serum-Eiweissfraktionen bei der elektrischen Aufladung des Blutserums

Serum	Datum	Aufladung	Relative %				A/G-Quot.	Gesamt-Eiweiss
			Albumin	α-Glob.	β-Glob.	γ-Glob.		
Fujii ♂ 30j.	5.3.52	0	52,1	7,3	14,0	26,6	1,09	7,2%
		−450 V.	43,4	7,8	10,8	38,0	0,76	
		+450 V.	51,5	7,4	11,8	29,3	1,06	
Matsui ♀ 23j.	20.6.52	0	57,5	3,9	7,0	31,6	1,35	7,3%
		−450 V.	55,7	5,6	9,6	29,1	1,25	
		+450 V.	56,3	6,0	9,2	28,5	1,29	
Hisatani ♀ 23j.	10.10.52	0	48,5	4,8	8,0	38,7	0,94	7,1%
		−450 V.	47,6	6,8	10,9	34,7	0,91	
		+450 V.	46,5	4,9	10,3	38,3	0,87	
Takeuchi ♀ 34j.	6.10.52	0	48,3	6,1	10,9	34,7	0,93	7,0%
		−450 V.	35,5	11,4	8,1	45,0	0,55	
		+450 V.	45,9	7,6	10,9	35,6	0,85	
Takagi ♂ 71j.	27.3.52	0	51,5	5,6	14,6	28,3	1,06	8,2%
		−450 V.	47,4	7,6	13,8	31,2	0,91	
		+450 V.	45,4	8,9	16,2	29,8	0,84	

mässigen Flz-Veränderung bei der Aufladung. Dagegen offenbart sich ein ganz enger Zusammenhang zum γ_2-Globulin, das sich bei der positiven Aufladung auf +450 Volt in weitaus der Mehrzahl der Fälle in einem neuen „peak" im Elektrophoresediagramm ausdrückt. Es ist jedoch nicht statthaft, eine bei der positiven Aufladung aufgetretene, zweite Zacke nun voreilig als eine γ_2-Fraktion zu deuten, da auch ohne Aufladung eine ähnliche Umgestaltung des letzten Gipfels der Gradienten eintreten kann, da an der Trennfläche ein T^+ bzw. T^- ∂- bzw. ε-Gradient, der ein Dichtesprung der Begleitsalzlösung ist, auftreten kann (vgl. Svensson)[39]. Abbildung 2 zeigt jedoch sehr deutlich, dass solche Bedenken nicht mehr bestehen, weil die Zwei-Höckerbildung der γ-Globuline bei positiver Aufladung ganz auffallend war. Die elektrophoretische Trennbarkeit der γ-Globuline hängt selbstverständlich von den Versuchsbedingungen, vor allem von der Wanderungsgeschwindigkeit im elektrischen Feld (bei pH 8,0, Phosphatpuffer) und dem Gehalt der γ_2- bzw. T-Fraktion, ab. Die Frage, ob die Verkleinerung des Albumin-Anteils, die Zunahme der γ-Globulinfraktion im Elektrophoresediagramm, und die Zu- oder Abnahme der α- und β-Komponenten, bei der elektrischen Aufladung des Menschen tatsächlich auf einer absoluten quantitativen Veränderung der Serumeiweiss-Anteile beruhen, oder einfach die Folge von der durch Verschiebung der Ionen stattfinden-

den Grössen-Aenderung der Brechungszahl der Serumproteine sein können, bleibt noch offen. Die letztere Ansicht schien aber eine gewisse Wahrscheinlichkeit für sich zu haben, da Scholtan[40] neuerdings darauf hinwies, dass der spezifische Brechwert der Eiweisstoffe von den verschiedensten Faktoren beeinflusst wird.

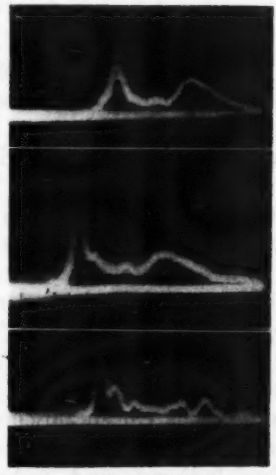


Abbildung 2. Auftrennung der γ-Globuline vom Serum einer Raynaudschen Krankheit in zwei Zacken.
Oben: Vor Aufladung. Mitte: −450 Volt. Unten: +450 Volt.

IV. Zusammenfassung

Fassen wir nun kurz unsere Ergebnisse über den Einfluss elektrischer Aufladung des Menschen und des Blutserums auf einzelne Serum- Eiweisskomponenten im Elektrophoresediagramm zusammen, so haben wir folgendes zu sagen:

1. In fast allen Fällen der Versuche nahm die Albumin-Fraktion mehr oder weniger deutlich ab und die γ-Globulinfraktion regelmässig zu, ganz gleich, ob es sich um eine positive oder um eine negative Aufladung handelte. Die quantitative Veränderung der α-und β-Globulinfraktion war nicht einheitlich. Man sieht also hier keine spezifischen Befunde.

2. Ganz charakteristisch und eindrucksvoll aber war die Auftrennung der γ-Globuline in zwei Unterfraktionen, d.h. in eine γ_1- und γ_2-Fraktion, die nur bei „positiver" Aufladung gelang, nicht dagegen bei negativer Aufladung.

3. Die Zustandsänderung der Gradienten kehrt schon binnen 30 Minuten nach beendeter Aufladung des Menschen wieder zum Ausgangsbilde vor der Aufladung zurück.

4. Die Veränderungen im Elektrophoresediagramm sind die Folge elektrostatischer Vorgänge, welche sich im menschlichen Organismus nur während der Aufladung abspielen und normalerweise unbewusst vor sich gehen.

5. Das Zustandekommen der elektrophoretischen Auftrennung der γ-Globuline in zwei Zacken gilt nicht nur für Patienten mit positiver, sondern auch negativer Takata-Reaktion.

6. Bei der positiven Aufladung des Blutserums in vitro tritt ebenfalls eine elektrophoretische Auftrennung der γ-Globuline in zwei Zacken ein, nicht dagegen bei der negativen Aufladung. Die hierbei entstandenen Veränderungen des elektrophoretischen Serumeiweissbildes sind nicht umkehrbar.

7. Auf Grund der früheren Feststellung, dass die Takata-Flockung hauptsächlich aus zwei Komponenten, d.h. Euglobulin und T-Fraktion, besteht, und in Anbetracht des elektrophoretischen Nachweises, dass die Takataflockungen bzw. Takata-Proteine mit den γ-Globulinen (γ_1 und γ_2) wandern, liegt es nahe, anzunehmen, dass die „T-Fraktion" (Takata) höchstwahrscheinlich mit dem γ_2 identisch sein müsse.

8. Aus Obigem geht hervor, dass die T-Fraktion (d.h. γ_2) sowohl theoretisch wie auch praktisch als ein spezifischer „Ladungsträger" bei der elektrischen Aufladung des Menschen fungieren muss.

9. Dem γ_1 (d.h. Euglobulinen) und anderen Komponenten der Serum-Eiweisse (d.h. Albumin und α- wie β-Globulinen) kommt eine Funktion, Träger der die Flockungszahl unmittelbar beeinflussenden Ladung zu sein, kaum oder gar nicht zu.

10. Die gesetzmässigen Flz-Veränderungen bei der elektrischen Aufladung des Menschen haben demnach lediglich mit der als Ladungsträger fungierenden T-Fraktion zu tun.

Literatur

1) Fujii, Tohoku J. Exp. Med. 1953, **59**, 105.
2) Takata, M., Helv. Med. Acta, 1950, **17**, 3, 254.
3) Takata, S., zit. nach Takata, M. (2).
4) Wuhrmann u. Leuthardt, Klin. Wschr., 1938, **12**, 409.
5) Wuhrmann u. Wunderly, Die Bluteiweisskörper des Menschen. Benno Schwabe Verlag, Basel 1947.
6) Edsall, Gilbert u. Scheraga, Abstracts Am. Chem. Soc., 112th Meeting, 33 C (New York, September, 1947). Zit. nach Pedersen (8).
7) Edsall, Fostet u. Scheinberg, J. Am. Chem. Soc., 1947, **69**, 2731. Zit. nach Pedersen (8).
8) Pedersen, Ann. Rev. Biochem. 1948, **17**, 169.
9) Deutsch, Alberty u. Gosting, J. Biol. Chem., 1946, **165**, 21.
10) Wallis, zit. nach Antweiler (11).
11) Antweiler, Die quantitative Elektrophorese in der Medizin. Springer-Verlag, Berlin-

Göttingen-Heidelberg 1952.
12) Smith u. Gerlough, J. Biol. Chem., 1947, **167**, 679.
13) Staub, Bruxelles-Médical, 1951, **48**, 2517.
14) MacLagan u. Bunn, Biochem. J., 1947, **41**, 582.
15) Olhagen, Acta Med. Scand. Suppl., 1947, **196**, 478.
16) Gohr, Falkenbach u. Langenberg, Zschr. gesamt. inn. Med., 1950, **5**, 407.
17) Gohr, Görges u. Feldmann, Zschr. gesamt. inn. Med., 1952, **7**, 926.
18) Albertsen, Christoffersen u. Heintzelmann, Acta Med. Scand., 1950, **136**, 302.
19) Eymer u. Zöllner, Deutsch. med. Wschr., 1949, 763.
20) Lasch u. Nowak, Med. Klin., 1952, **47**, 69.
21) Takata, S., Fujii u. Otani, Nippon Shokakibyo Gakkai (Kongr.), Tokyo 1951.
22) Wakamatsu, Nippon Naika Gakkai (Kongr.), Kanazawa 1949.
23) Nabekura, Seibutsu Butsuri-Kagaku (Jap.), 1951, **1**, 71.
24) Yoshida u. Sumitomo, Seibutsu Butsuri-Kagaku (Jap.), 1952, **1**, 133.
25) Fujiyama, Seibutsu Butsuri-Kagaku (Jap.), 1952, **1**, 133.
26) Nomura, Nippon Shokaki-byo Gakkai Zasshi, 1951, **49**, 66.
27) Nomura, Nippon Naika Gakkai Zasshi (Jap.), 1953, **42**, 134.
28) Ueda, Nippon Naika Gakkai u. Nippon Shokaki-byo Gakkai (Kongr.), Tokyo 1953.
29) Takata, M. u. Hatashita, siehe Takata, M. (31).
30) Takata, M. u. Takata, S., Sei-Kagaku (Biochemie), 1952, **24**, 1.
31) Takata, M., Die Medizinische, 1952, 617.
32) Takeuchi, Nippon Geka Gakkai (Kongr.), Tokyo 1953.
33) Wiedemann, Schweiz. med. Wschr., 1946, **76**, 241.
34) Wuhrmann, Wunderly u. de Nicola, Klin. Wschr., 1950, **28**, 667.
35) Wuhrmann, Schweiz. med. Wschr., 1952, **82**, 937.
36) Emmrich, Das Bluteiweissbild, Diagnose und Therapie der Bluteiweiss-Störungen. Ferdinand Enke Verlag, Stuttgart 1952.
37) Dole, J. Clin. Invest., 1944, **23**, 708. Zit. von Knedel (38).
38) Knedel, Arzneim.-Forsch., 1952, **2**, 182.
39) Svensson, Ark. Kem. Mineralog. Geol., 1945, **21**, No. 5.
40) Scholtan, Theoret. Med., 1952, **6**, I/147.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

Studies of Tuberculous Pus by Means of Paper Chromatography

Second Report

By

Takuo Tamaki
(玉置 拓夫)

From the Department of Orthopedic Surgery, Faculty of Medicine, Tohoku University, Sendai; Director: Prof. S. Iino

(Received for publication, June 22, 1953)

In this Journal in 1952,[1] I have reported for the first time on my study on the amino acids from the protein and free amino acids in tuberculous pus plasma, wherein I stated that I could not prove existence of cystine and methionine in the composition in the amino acids from the protein in such pus plasma, and that free amino acids do not come forth in pus from abscesses not previously punctured, but are found existing in pus from tuberculous abscesses frequently punctured previously. In this second report, I will publish the results of my follow-up study with 44 samples of tuberculous pus and 4 samples of nontuberculous pus as control, as material, and state my findings on the free amino acids, the composition of the amino acids obtained from the protein in pus plasma, and in particular, on my search for the existence of cystine and methionine.

Experimental

Method

The same method as previously reported[2] has been followed. For the detection of cystine and methionine, I used, beside the method of preliminarily oxidizing these compounds into cystic acid and methionine sulfoxide with 30% H_2O_2 water,[3] the coloration method with potassium iodoplatinate,[4] concurrently.

Results

1) Amino acids composing protein in pus plasma

I have previously reported on the appearance of 13 spots corresponding to aspartic acid, glutamic acid, serine, glycine, alanine, tyrosine, valine, proline, leucine, phenylalanine, lysine, histidine and arginine in the chromatogram of 3 samples of hydrolyzed protein from tuberculous pus

plasma taken from closed iliac abscesses of spondylitis tuberculosa patients. Then, I failed to prove the existence of cystine and methionine, but as the failure might have been due to the minuteness of the quantity of these components even below the minimum detectable quantity, when treated under a method adapted for detection of the other amino acids, I made a minute reexamination, with this possibility in mind, and succeeded in isolating and fixing cystic acid and methionine sulfoxide. It is noteworthy that of the 13 spots mentioned above, the spot corresponding to proline showed the relative largeness of its quantity.

2) Free amino acids in pus plasma

TABLE I

Free Amino Acids in the Protein-free Extracts of Cold Pus Plasma from Abscesses never punctured before

No.	Free amino acid
1	
2	
3	
4	
5	
6	Alanine (faint), leucine.
7	
8	Glutamic acid, alanine, valine or methionine, tyrosine, phenylalanine, leucine.
9	Serine, glycine, alanine, valine or methionine, tyrosine, leucine.
10	
11	
12	Valine or methionine, phenylalanine, leucine, histidine.

NOTE: The statement valine or methionine shows that it has been impossible to separate the two spots for these compounds due to their natural overlapping.

i) Out of the 12 samples of cold pus from abscesses never punctured before, 4 were found to contain free amino acids, in a frequency in the following order: Leucine, valine or methionine, alanine, tyrosine, phenylalanine, glutamic acid, serine, glycine and histidine.

Two of the 4 positive cases were found to contain 6 kinds of free amino acids in total of the above enumerated 9.

ii) Out of 23 samples of cold pus from abscesses often punctured before, 21 were found positive in free amino acids, as shown in Tab. II below, comprising the 14 kinds of valine or methionine, alanine, glutamic acid, phenylalanine, leucine, tyrosine, aspartic acid, proline, glycine, hydroxyglutamic acid, serine, threonine, tryptophane and histidine. Fourteen of the positive samples contained less than 4 kinds of free amino

TABLE II

Free Amino Acids in the Protein-free Extracts of Cold Pus Plasma from Abscesses often punctured before

No.	Free amino acid
1	Aspartic acid, glutamic acid.
2	Aspartic acid, glutamic acid, alanine.
3	Aspartic acid, glycine, alanine.
4	Alanine, valine or methionine.
5	Glycine, valine or methionine.
6	Glutamic acid (faint), alanine.
7	Glutamic acid, alanine, valine or methionine, proline, leucine.
8	
9	Valine or methionine, leucine.
10	Glutamic acid, alanine, valine or methionine, tyrosine, leucine.
11	Glutamic acid, alanine, valine or methionine, tyrosine, proline, phenylalanine, leucine, hydroxyglutamic acid.
12	Aspartic acid, valine or methionine.
13	Alanine (faint), valine or methionine (faint), phenylalanine (faint).
14	Serine, glycine, valine or methionine, phenylalanine, leucine, threonine, hydroxyglutamic acid, histidine (faint).
15	Alanine (faint), valine or methionine, tyrosine, proline, phenylalanine, leucine.
16	Glutamic acid, alanine, valine or methionine, tyrosine, phenylalanine, leucine.
17	Glutamic acid, alanine, valine or methionine, tyrosine, phenylalanine, leucine.
18	Glutamic acid, alanine, valine or methionine, tyrosine, phenylalanine, leucine, tryptophane.
19	Glutamic acid (fait), alanine (faint), valine or methionine.
20	
21	Alanine (faint), valine or methionine (faint), phenylalanine (faint).
22	Glutamic acid (faint), alanine (faint), valine or methionine (faint), phenylalanine (faint).
23	Glutamic acid, valine or methionine, tyrosine, proline, phenylalanine, leucine.

acids, inclusive, and the other 9 samples not less than 5.

iii) The nine smaples of tuberculous pus from abscesses with sinus were found containing free amino acids without exception, as shown in Tab. III, in the order of frequency of leucine, phenylalanine, valine or methionine, alanine, glutamic acid, tyrosine, glucosamine, aspartic acid, proline and α-amino-capric acid. Out of the total of 9 cases, the samples from the 2 cases who were proved free of secondary infection contained only one or two kinds of amino acids, but the other 7 samples from cases with proved secondary infection contained each more than 5 kinds of free amino acids.

3) Free amino acids in non-tuberculous pus.

TABLE III

Free Amino Acids in the Protein-free Extracts of Tuberculous Pus Plasma with Sinus

No.	Free amino acid
1	Valine or methionine, phenylalanine, leucine, α-amino capric acid ? glucosamine.
2	Glucosamine.
3	Alanine, glucosamine.
4	Alanine, valine or methionine, tyrosine, phenylalanine, leucine.
5	Aspartic acid, alanine, phenylalanine, leucine, α-amino capric acid ?
6	Aspartic acid, glutamic acid, alanine, valine or methionine, phenylalanine, leucine.
7	Alanine, valine or methionine, proline, leucine.
8	Glutamic acid, valine or methionine, tyrosine, phenylalanine, leucine.
9	Valine or methionine, proline, tyrosine, phenylalaline, leucine.

TABLE IV

Free Amino Acids in the Protein-free Extracts of non-tuberculous Pus Plasma

No.	Free amino acid
1	Aspartic acid, glutamic acid, alanine, valine or methionine, tyrosine, phenylalanine, leucine.
2	Serine, glycine, alanine, valine or methionine, tyrosine, phenylalanine, leucine, threonine, histidine, lysine, arginine.
3	Glutamic acid, alanine, valine or methionine.
4	Glutamic acid, alanine, valine or methionine, tyrosine, phenylalanine, leucine.

In all the 4 samples of such pus used as control, not less than 6 kinds of free amino acids were proved, except in the one sample too small in quantity for the detection of such ingredients. In all, 13 kinds of amino acids, namely, alanine, valine or methionine, glutamic acid, tyrosine, phenylalanie, leucine, aspartic acid, serine, glycine, threonine, histidine, lysine and arginine, were found in existence.

CONCLUSION

1) By means of chromatographic study, it is found that the varieties of amino acids contained in the hydrolyzed protein in tuberculous pus plasma is not particularly dissimilar to those in the hydrolyzed protein in blood plasma.[5] Only the relative abundance of proline and the paucity in cystine and methionine in the former protein makes its chromatogram closely resemble that of γ-globuline.[6]

2) Free amino acids are not widely found in pus from abscesses free from previous punctures but in most of the samples of pus from frequent-

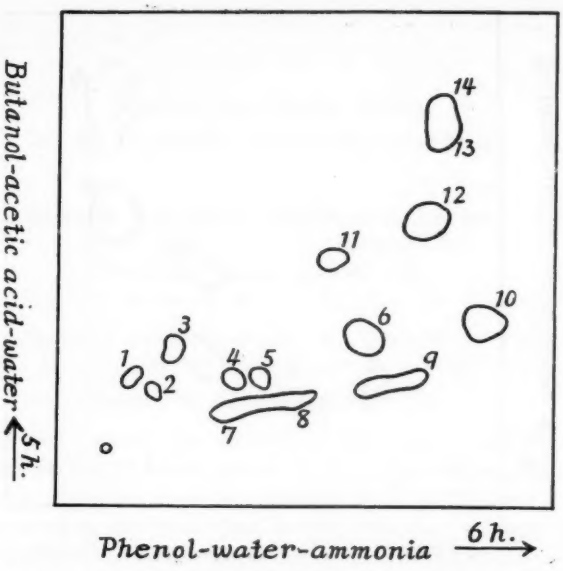


Fig. 1. Butanol-acetic acid-water/phevol-water chromatogram of the complete hydrolysate of the tuberculous pus plasma protein developed with ninhydrin (temp., 20°C).

1: Cysteic acid 2: Aspartic acid 3: Glutamic acid 4: Serine 5: Glycine 6: Alanine 7: Lysine 8: Histidine 9: Arginine 10: Proline 11: Tryosine 12: Valine or Methionine 13: Leucine 14: Phenylalanine

ly punctured abscesses, and in all samples of pus taken from abscesses with sinus without exception. In this respect, my previous assertion that no free amino acid was to be found in pus from previously unpunctured abscesses is a misstatement due to premature judgment.

On the other hand, tubercle pus may be classified into two categories —the one containing no or only a few free amino acids, with weak proteolysis, and the other containing many varieties of free amino acids due to strong proteolysis and showing no essential difference from non-tuberculous hot pus in this respect.

In this meaning, Müller's opinion[7] that the essential difference of tubercle pus from non-tuberculous pus consists in the fact that the former is rarely found to contain free amino acids and no tyrosine at all is found untenable.

References

1) Tamaki, Tohoku J. Exp. Med., 1952, **55**, 247.
2) Yoshizawa, Z., *ibid.*, 1950, **53**, 125.

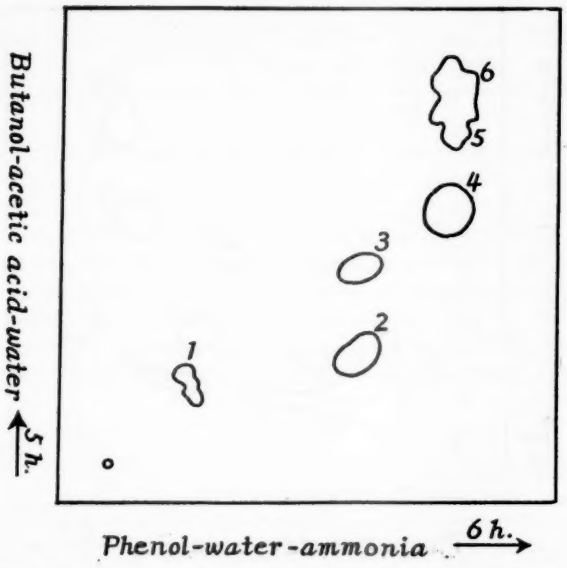


Fig. 2. Butanol-acetic acid-water/ phenol-water chromatogram of the tungstic acid filtrate of the tuberculous pus plasma from abscess had been punctured before developed with ninhydrin (temp., 20°C).

1: Glutamic acid 2: Alanine 3: Tyrosine 4: Valine or Methionine 5: Leucine 6: Phenylalanine

3) Dent, Biochem. J., 1949, **44**, 610.
4) Block, Winegard & Toennis, Science, 1948, **108**, 506.
5) Yamamura, Iryo (Jap.), 1950, **4**, 13.
6) Brand, Ann. New York Acad. Sci., 1946, **57**, 218.
7) Müller, Cbl. inn. Med., 1907, **28**, 297.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

Studies on Brain Stem

V. On the Location of Facial Nucleus of Birds

By

Shigeaki Yamamoto, Mitsuyoshi Oikawa,
(山本 重亮) (及川 光榮)
Toshiaki Konno and **Ko Ohi**
(今野 利秋) (大 井 康)

From the Anatomical Laboratory of Prof. H. Seto, Faculty of Medicine, Tohoku University, Sendai

(Received for publication, June 30, 1953)

Yamamoto,[1][2] one of the authors of this paper, has reported previously on the nuclei of facial nerves in mammals belonging to various genera, human embryos of all the months in fetal life, and also in an anencephalus, and made clear that in not one case of these there was any trace of displacement according to the neurobiotaxis theory of Kappers.

According to Kappers,[3][4] the facial nucleus, both ontogenetically and phylogenetically, is said to change its position, first from the cranial to the caudal, and next, from the dorsal to the ventral sides, in compliance with the law of neurobiotaxis. In the above, the influence of the pyramidal tract is postulated for the displacement from the dorsal to the ventral side, but as the pyramidal tract is absent in birds, there could be no displacement of the facial nucleus by birds from the dorsal to the ventral side. Kappers[5] further discusses the influence of the reflex centers on the displacement of the nucleus. The following study was made to clarify these points of doubt.

Experimental

Materials

As materials, series of Pal-carmine-stained transverse sections of the brains of a pigeon, a japanese bush-warbler, a baby crow, a gull, an owl and a cock were used.

Findings

Pigeon. The facial nucleus seems as located considerably on the dorsal side, as indicated by its position against the ventral border of the brain stem, but when the relative position to the trapezoid body is considered, it is found in nearly the same position as in mammlas. In

mammals, the trapezoid body makes the boundary of the tegmental part and the basilar part of the pons running transversely between them, and in birds, the situation is quite similar. Only, in birds, the basilar

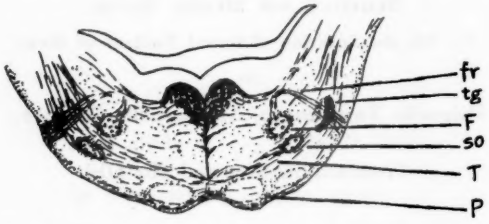


Fig. 1. Transversal section of brain stem in pigeon, Pal-carmine staining, low power view. F facial nucleus; fr facial root fibres; tg descending trigeminal root; T trapezoid body; so superior olive; P pontal nucleus.

part is weaker in development than in mammals, but the pontal nucleus is in a relatively strong development, forming a definite mass in birds, while it is represented by some scattered cell groups in mammals. In view of this fact, we have taken the trapezoid body as our standard to determine the position of the facial nucleus in the tegmentum of birds. The relative position of the facial nucleus to the nucleus of the descending trigeminal root is also quite analogous to that in mammals, and no specific displacement toward the latter is noticeable. The intrapontine facial nerve turns ventrolaterally, after a short ascending course, and parts from the brain stem, as is the case with mammals, but it differs from the counterpart in mammals in that the pars 1 is shorter and no genu is formed in the course. The nucleus fasciculi solitarii is more in the caudal side of the facial nucleus and on a different level.

Japanese bush-warbler. The facial nucleus is situated directly on the dorsal side of the trapezoid body, as in the case of pigeon, and is not divided into the dorsal and ventral nuclei. The intrapontine facial nerve has scarcely an ascending part (pars 1) nor a genu, and goes out ventro-laterally in an arc, immediately upon emerging from the nucleus.

Baby crow. The facial nucleus is quite in the normal position. From its position relative to the ventral border of the brain stem, it seems as located considerably on the dorsal side, but not so in relation to the trapezoid body. The ascending part of the intrapontine facial nerve is even shorter than in the brain of a bush-warbler, and without describing an arc, runs straightly ventro-lateral-wards. Of course no genu is formed. The nucleus makes its appearance as soon as the descending trigeminal root disappears, on its ventral side.

Gull. The facial nucleus appears far up in the cranial side of the

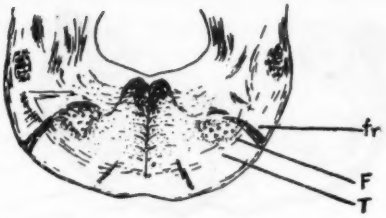


Fig. 2. Transversal section of brain stem in japanese bush-warbler, Pal-carmine staining, low power view. Facial root fibres fr have scarcely an ascending part nor a genu. F facial nucleus; T trapezoid body.

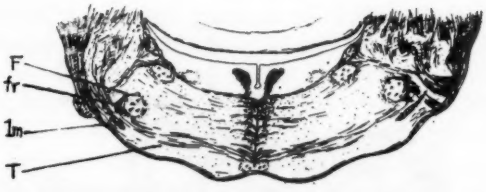


Fig. 3. Transversal section of brain stem in baby crow, Pal-carmine staining, low power view. F facial nucleus; fr facial root fibres; lm lateral lemniscus; T trapezoid body.

nucleus fasciculi solitarii. It is divided into the dorsal and the ventral parts, of which the latter is buried in the trapezoid body, while the former is situated a little more on the dorsal side than in mammals, but not in a perceptible degree. It is not at all displaced toward the descending trigeminal root. The intrapontine facial nerve has an ascending part, but forms no genu.

Owl. The facial nucleus appears on the cranial side of the nucleus fasciculi solitarii and is divided into the two parts of dorsal and ventral. The former part appears on a level a little more cranial than the latter, and also is extended more cranially. In development, the ventral part is far stronger, and extends to the vicinity of the ventral border of the brain stem, in the trapezoid body. To the descending trigeminal root it stands in the same relative position as in the above specimens. The intrapontine facial nerve is strongly developed in its ascending part, but no genu is formed.

Cock. The facial nucleus is located far to the cranial of the nucleus fasciculi solitarii and is divided in the ventral and the dorsal parts. In examining the series of slides starting from the caudal end, the ventral nucleus appears slightly later than the dorsal nucleus but remains in sight much longer. In development, the ventral part is stronger, generally

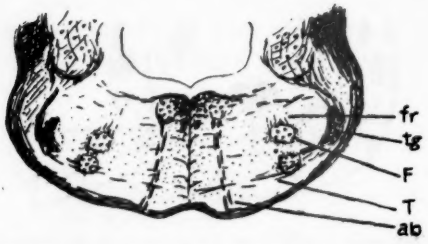


Fig. 4. Transversal section of brain stem in gull, Pal-carmine staining, low power view. F ventral and dorsal parts of facial nucleus; fr facial root fibres; tg descending trigeminal root; T trapezoid body; ab abducens nerve.

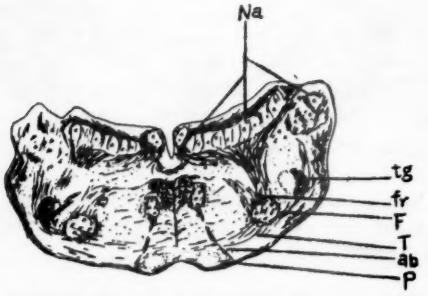


Fig. 5. Transversal section of brain stem in owl, Pal-carmine staining, low power view. F facial nucleus; fr facial root fibres which form a remarkable ascending part; tg descending trigeminal root; T trapezoid body; Na Nucleus VIII; P pontal nucleus; ab abducens nerve.

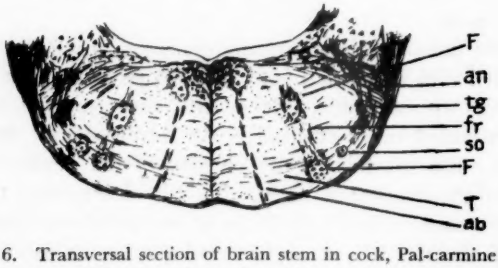


Fig. 6. Transversal section of brain stem in cock, Pal-carmine staining, low power view. F facial nucleus; fr facial root fibres; so superior olive; tg descending trigeminal root; T trapezoid body; ab abducens nerve; an acoustic nerve.

speaking, but on the level where both are in fullest development, the two parts are of nearly equal development. The ventral nucleus is near the ventral border of the brain stem, and is rather on the ventral side of

the trapezoid body, while the dorsal nucleus is located considerably on the dorsal side, being almost on the same level with the descending trigeminal root. However, both the nuclei are at the normal distance from the descending trigeminal root as in other cases and no displacement to a nearer vicinity thereto is observable. The root fibres originating in the nuclei form an ascending part but no genu.

Discussion

As stated in the Introduction, according to the neurobiotaxis theory by Kappers, the facial nucleus, both ontogenetically and phylogenetically, should undergo displacement first from the cranial to the caudal and then from the dorsal to the ventral side, the first displacement being due to the influence of the nucleus fasciculi solitarii, a nucleus of taste, the genu in the intrapontine facial nerve being formed thereby. In a study on various mammals, Yamamoto, one of the authors of this paper, has found that the facial nucleus appeared first about the same time with the disappearance of the nucleus fasciculi solitarii, when the series of brain sections are examined upward from the caudal end. In birds, however, the fasciculus solitarius and its nucleus seem to be somewhat weaker than in mammals, though completely developed, and the facial nucleus is located at a long distance to the cranial side and its root fibres form no genu. If Kappers' theory is acceded to, this fact shows that the displacement to the caudal side did not occur by birds, but seeing that the nucleus fasciculi solitarii is in full development, it is incomprehensible how the caudal displacemant could have failed to occur. If the neurobiotaxis theory is well-founded, why should there be no trace at all of such a displacement, namely, a formation of genu, in facial nerve root in birds' brain as well as in mammals?

Again, as stated above, as the pyramidal tract is believed to be missing in the brain by birds, it is impossible by them that the facial nucleus should be displaced to the ventral side from the dorsal, owing to the impulse from the pyramidal tract. Then, the facial nucleus must be in a position far more to the dorsal side than in mammals. But when the trapezoid body that runs along the boundary of the basilar and tegmental parts of the pons is taken as the base-line, the facial nucleus in birds is situated much in the same position as in mammals and not more to the dorsal. In a cock, indeed, it is on the ventral side of the trapezoid body, almost touching the border of the brain stem, so in a position more to the ventral than in mammals. In a gull and an owl also, the facial nucleus is found buried in the trapezoid body and considerably on the ventral side. This fact is at variance with the deduction from the neurobiotaxis theory by Kappers. In fact, by a gull, an owl and a cock, there are two nuclei

in the facial nucleus, the ventral and dorsal, and the dorsal is situated somewhat in a dorsal position, as compared with the facial nucleus of mammals, but if the facial nucleus has undergone displacement at all, it is beyond understanding how the two parts of the same nucleus should have behaved differently. Another deduction from the neurobiotaxis theory will have it that the pars 1 of the intrapontine facial nerve has been formed by the ventralward displacement of the facial nucleus, but then, how can it be that there is no formation of pars 1 in a bush-warbler and a baby crow, though in a pigeon, a gull, an owl and a cock it is developed in some degree. Such a diversified result obtained with various birds which are all deficient in pyramidal tract seems to be one of the proofs of the inadequacy of the neurobiotaxis theory.

The birds, with no pyramidal tract, have to rely on their extrapyramidal tract for movement, and the part played by the rubrospinal, the tectospinal and the vestibulospinal tracts comprised in the extrapyramidal tract must be more important than in mammals. According to the neurobiotaxis theory, the facial nucleus in birds should show some reaction to these tracts, but there is no displacement toward these tracts observable. It may be, as some of these tracts are distributed on the opposite sides as viewed from the facial nucleus, the latter is inhibited from moving to any one side.

Furthermore, Kappers emphasizes that the trigeminal nucleus exerts a strong influence on the facial nucleus, so that the latter is displaced to the former, or laterally. In birds, where there is no pyramidal tract to influence the position of the facial nucleus, the influence of the trigeminal nucleus must be even felt stronger, according to his contention, but our observations have not revealed any perceptible displacement of the facial nucleus toward the trigeminal nucleus, particularly toward the nucleus of the descending root of trigeminal nerve, in comparison with the observation on the brains of mammals. The relative position of the two nuclei, if we take the superior olive as standard, as Kappers has done, are somewhat unanalogous from bird to bird, and we hope we shall have another opportunity to report thereon. From the above, it is clear that the neurobiotaxis theory does not hold in the anatomy of birds, as little as in the case of mammals.

Summary

1. Our study was conducted with series of Pal-carmine stained transverse sections of the brains of a pigeon, a japanese bush-warbler, a gull, a baby crow, an owl and cock as materials.

2. In a bird's brain, the fasciculus solitarius is fully developed, but the facial nucleus is located far up in a cranial position, and there

is no trace that it has ever moved down caudally.

3. In a bird's brain, the pyramidal tract is lacking altogether, yet the facial nucleus is generally situated in much the same position as occupied by it in mammals, the pars 1 of intrapontine facial nerve being found formed in some cases only.

4. There has been no observation revealing that the facial nucleus has been particularly drawn toward the extrapyramidal tract.

5. Concerning the question whether the facial nucleus is displaced toward the nucleus of the descending trigeminal root, it may be mentioned that the relative position of the two organs above, if the position of the superior olive is taken as standard, is somewhat unlike from bird to bird.

6. In consideration of the above, we find it impossible to recognize the applicability of the neurobiotaxis theory in the case of birds as little as in the case of mammals.

References

1) Yamamoto, Tohoku J. Exp. Med., 1952, **56**, 331.
2) Yamamoto, *ibid.*, 1952, **56**, 339.
3) Kappers, Neurol. Zentralbl., 1907, **26**, 834.
4) Kappers & Vogt, Neurol. Zentralbl., 1908, **27**, 958.
5) Kappers, The comparative anatomy of nervous system of vertebrates, Vol. 1, Macmillan, New York, 1936.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

Quantitative Measurement of Decreased Bone Conduction Acuity with Occluded External Auditory Canal

By

Tokuro Suzuki and **Norio Ichikawa**
(鈴木 篤郎) (市川 則雄)

From the Department of Otolaryngology, Faculty of Medicine, Shinshu University, Matsumoto

(Received for publication, July 1, 1953)

It is an already accepted fact that, with the normal ear, sensitivity to bone conducted sound increases when the external auditory canal is occluded.

According to Kelley and Reger[1] in 1937, this fact had been reported by Wheatstone as early as in 1827.

This increase of sensitivity to bone conducted sound with the external ear canal occluded can be observed only in the ear with normal sound conduction system, while it is not noticeable in the ear with sound conduction disturbance.

For this reason, this type of examination is employed to distinguish perceptive deafness from conductive deafness. This is what is known as Bing Test.

Of late, the audiometer has been in use for Bing Test for the purpose of precise quantitative examination.

According to the reports of Aubry, Causse, and Chavanne[2] in 1938, Watson[3] in 1943, Lierle and Reger[4] in 1946, Sullivan, Gottlieb, and Hodges[5] in 1947, and Onchi[6] in 1951, with the normal ear and the ear with perceptive deafness, sensitivity to sound of below 1000 cps increases from 15 to 20 db more when the external canal is occluded than when it is unoccluded, while with the ear having even slight conductive impairment, such increase in sensitivity is not observed. Thus, occluded bone conduction test is being used as one of the most effective means of discovering conductive deafness.

We have been trying to apply this occluded bone conduction test to perform quantitative measurement of decreased bone conduction acuity. This method of testing is found to have the following advantages over the usual bone conduction test carried out with the external auditory canal open:

1. The first advantage is that the loss of hearing measured by occluded bone conduction test can be considered as to indicate directly the degree of perceptive deafness. In occluded test the phenomenon of "increased bone conduction acuity" in conductive deafness disappears. This can be easily imagined from the fact that sensitivity of a normal ear to bone conducted sound increases when the canal is closed, while that of an ear with conductive deafness does not increase. This was quantitatively proved by the authors through observations described below:

Tested were 31 normal ears and 36 ears clinically diagnosed as having conductive deafness (deafness caused by otosclerosis excluded).

Bone conduction thresholds were tested on these ears occluding the external auditory canal with a piece of cotton dipped in glycerin, the opposite ear not being tested being masked with white noise of 40 db. The results were as shown in the Table below:

TABLE I

Average of Occluded Bone Conduction Threshold of 31 Normal Ears and 36 Ears with Conductive Deafness

Frequency	256	512	1024	2048	4096
Normal ears	40.5 db	30.5 db	22.1 db	29.2 db	44.7 db*
Ears with conductive deafness	45.3 db	33.6 db	27.0 db	33.5 db	48.6 db
Differences	4.8 db	3.1 db	4.9 db	4.3 db	3.9 db

* Audiometer dial reading without calibration.

Upon comparing these two groups, the normal ears showed better ensitivity to any frequency range tested, and it became clear that this difference was significant in statistical examination. However it is not certain whether this difference essentially existed between the normal ear and that with conductive deafness, or it was noticed because there might have been some element of perception system disturbance in what had been clinically diagnosed as pure conductive deafness. Inasmuch as the difference of thresholds between these two groups was so little that it did not reach over 5 db, in occluded bone conduction test, the threshold of the normal ear and that with conductive deafness can be regarded as the same, from clinical point of view.

Accordingly, in occluded bone conduction test, if the threshold of an ear with deafness is shifted higher than that of a normal ear, the degree of this shifting can be regarded as the degree of perceptive deafness.

It is reported that sensitivity to bone conduction may decrease in the case of conduction system disturbance from otosclerosis, so this will

have to be considered separately.

2. The second advantage is that the examination can be made just as precisely in a room without sound-proof set up. When a bone conduction test is given with the canal open, the ear is apt to receive more masking effect of the room noise than in air conduction test. However, when a test is made occluding the canal, sensitivity of the normal ear to air conduction decreases as shown in Fig. 1 which indicates that masking effect of room noise is much less than a test with the canal open. This is a proof that a test can be made just as precisely in any test room.

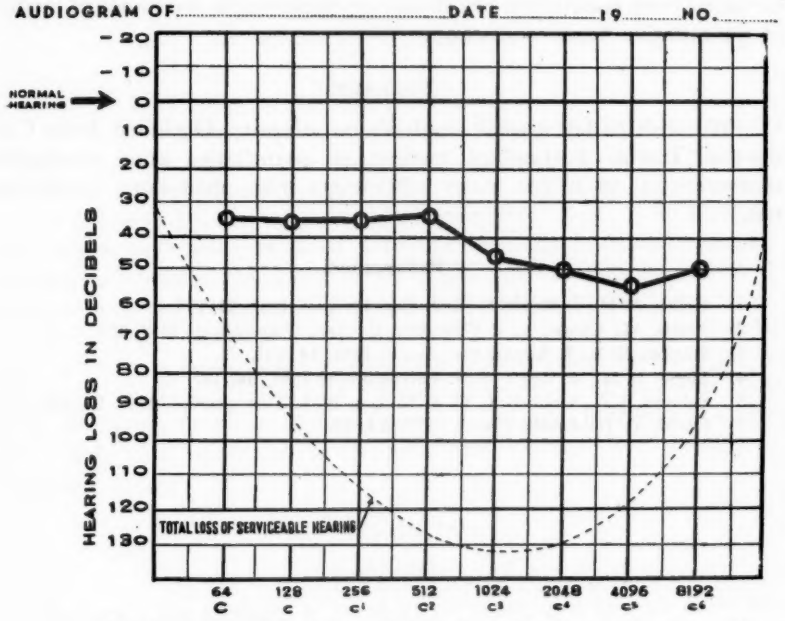


Fig. 1. Average shift of air conduction threshold of seven normal ears with external auditory canal occluded.

3. The third advantage is that radiation of a bone conduction receiver can be prevented from being conveyed to the ear through air conduction. With some bone conduction receivers, sound is audible through air conduction much better than through bone conduction when they are tuned over 2000 cps. This defect of a receiver will have to be improved by the manufacturer, but such inconveniences can be entirely done away with when a receiver is applied to the ear with the external auditory canal occluded.

4. The fourth advantage is that the testable range of hearing loss of a ear with perceptive deafness can be made wider by this test.

When the external ear canal of a normal ear or an ear with perceptive deafness is occluded, sensitivity to bone conducted sound increases from 15 to 20 db with a sound below 1000 cps, so the testable range of the audiometer can be made wider in proportion.

As is described above, the test of measuring bone conduction threshold by occluding the external ear canal has various advantages over the usual test with open canal in quantitative measurement of decreased bone conduction acuity. Therefore, we have come to the conclusion that occluded bone conduction test should be employed as the standard method of quantitative bone conduction.

Summary

We wish to lay special emphasis on adopting Occluded Bone Conduction Test as a standard method of quantitative bone conduction measurement, as it has many advantages over open bone conduction test.

References

1) Kelley, N. H., & Reger, S. N., J. Exp. Psychol., 1937, **21**, 211.
2) Aubry, M., Causse, J., & Chavanne, L., Ann. d'oto-laryng., 1938, 689.
3) Watson, N. A., J. Acoust. Soc. Amer., 1934, **14**, 207.
4) Lierle, D. M., & Reger, S. N. Laryngoscope, 1946, **56**, 187.
5) Sullivan, J. A., Gottlieb, C. C. & Hodges, W. E. Laryngoscope, 1947, **57**, 690.
6) Onchi, Y. Jibi-Inkoka (Jap.), 1952, **24**, 93.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

The Influence of Various Narcotics on Cerebral Circulation

By

Naohiko Tokita
(鴇田 尚彦)

From the Surgical Clinic of Prof. S.-T. Katsura, Faculty of Medicine, Tohoku University, Sendai

(Received for publication, July 1, 1953)

Introduction

When various narcotics are used for general anesthesia, it is important to know how the respiration and cerebral circulation changes its status during the stages of anesthesia.

It has been known for years that the cerebral cortex is first affected with inhalation anesthesia, then the spinal cord and medulla oblongata become anesthetized in the order. It is also interesting to know how the circulation in the cerebrum and the medulla oblongata changes.

Since Pick[1] classified anesthetics into two groups, *i.e.*, cortical and subcortical, it is interesting to know how these drugs influence upon the circulation in the cerebrum and medulla oblongata. In order to clarify these interesting problems, cat was used to study the influence of various kinds of narcotic upon the circulation in the cerebrum and medulla oblongata.

Experimental

Method

Recently, the changes of cerebral circulation in human have been studied with N_2O method. This method is, however, very difficult to study the cerebral circulation during inhalation anesthesia and it is impossible to measure the blood flow continuously. Several other methods to study the cerebral circulation have been reported. In order to study the local changes in cerebrum and medulla oblongata, thermocouple by Schmidt[2] is a convenient method. The author adopted this method and recorded the swing of the galvanometer with the respiratory curve by a long recording camera. Adult cats weighing 2.5–3.0 kg. were strapped to a frame on prone position. The head was especially fixed tightly to prevent even a slight motion during the course of experiment without

pressing upon the neck. After local anesthesia, trepanation was made in the frontal region of the head opening a hole as large as the tip of index finger. After dura mater was opened, gyrus postcruciatus was exposed and the attachement of the thermocouple was pierced 1–2 mm. into the brain avoiding large blood vessels and fixed. The attachement was thrust into the medulla oblongata through the cisterna magna. A cathether was introduced into the trachea through tracheotomy in all cases to secure the patency of the airway. A funnel was attached to one end of the endotracheal catheter and a gauze was placed upon it. Open drop method was used over the gauze during inhalation anesthesia. A vein in the lower extremity was exposed for intravenous anesthesia and the drugs were injected. A manschette was wrapped around the chest to record the curve of respiration. A thermometer was placed in the anus to check the changes of body temperature. In order to prevent the change of body temperature, the body was wrapped with a cloth and a 40 watt electric bulb was used to keep the animal warm. For inhalation anesthetics, ether, chloroform, and ethyl chloride were used. Oltopan Sodium (Evipal Sodium), and Amybal (Amytal Sodium), were used as intravenous narcotics. 2% morphine hydrochloride was used for analgesics.

Results

Control group

Bilateral carotid arteries were tied simultaneously in 2 cases. As shown in Fig. 1, the curve of cerebral circulation showed a decline. However, after the release of the ligature, the curve showed a wave-like changes

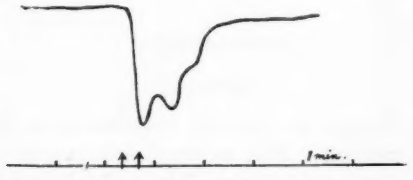


Fig. 1. Change of cerebral blood flow due to simultaneous ligature of bilateral carotid arteries. The first arrow shows the ligature, the second shows the release.

and returned to normal in about 2 minutes. According to Yamakita,[3] Schmidt,[2] and Jasper,[4] adrenalin is thought to cause a transient rise of blood pressure and increase the cerebral circulation. 1.0 cc. (1:10,000) solution was injected intravenously and a sharp temporary elevation of the circulation curve was observed (Fig. 2).

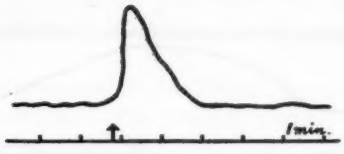


Fig. 2. Cerebral blood flow due to adrenalin injection. Arrow shows the intravenous injection.

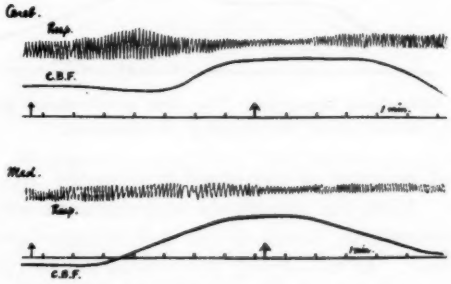


Fig. 3. Ether anesthesia. Upper part is cerebrum (cereb.), lower part is medulla oblongata (med.).

Resp., respiration curve. C.B.F., cerebral blood flow. The first arrow shows the start of anesthesia, the second shows the stoppage of anesthesia.

Ether (*Inhalation anesthesia*) (Fig. 3)

Ether was used in 12 cases. All curves of the circulation in the cerebrum and medulla oblongata were almost identical except in 2 cases. The curve showed a very slight decline 30 seconds—2 minutes after the inhalation anesthesia, then the circulation started to increase 2–3 minutes later, and the curve was still ascending in the stage of deep anesthesia. 13–20 minutes after the beginning of the inhalation, the anesthesia reached the 3rd–4th plane of the 3rd stage and the circulation was at the maximum. When the anesthesia was stopped, the curve returned to the preanesthetic condition within 8–30 minutes.

Of the 2 cases which showed exceptional curve, 1 case showed a slight decrease of the blood flow in the cerebrum and medulla oblongata, while, in the other case, the cerebral circulation in the cerebrum decreased in spite of a slight increase in the medulla oblongata.

Chloroform (*Inhalation anesthesia*) (Fig. 4)

12 cases were stndied in this experiment. 10–20 seconds after the beginning of inhalation, the blood flow increased in the cerebrum and medulla oblongata. 3–4 minutes later, the blood flow reached the maximum curve during the deep anesthesia. If the anesthesia was still continued,

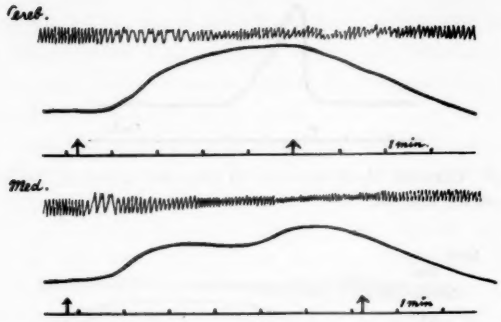


Fig. 4. Chloroform anesthesia. The first arrow shows the start of anesthesia, the second shows the stoppage of anesthesia.

some cases showed decrease of the blood flow in the 3rd—the 4th plane of the 3rd stage. After the anesthesia was discontinued, the curve returned to the preanesthetic status within 5–6 minutes and few cases still continued to decline. In two cases, the respiration stopped 1–2 minutes after the beginning of the anesthesia showing rapid decrease of blood flow in the cerebrum and medulla oblongata. Immediately after anesthesia had been stopped and artificial respiration started, the blood flow recovered. However, it decreased after natural respiration started.

Ethyl chloride (*Inhalation anesthesia*) (Fig. 5)

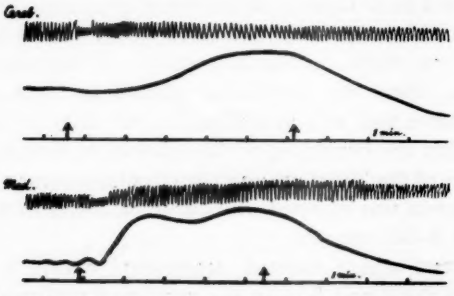


Fig. 5. Ethyl chloride anesthesia. The first arrow shows the start of anesthesia, the second shows the stoppage of anesthesia.

4 experiments were done. In 3 cases, the blood flow in the cerebrum and medulla oblongata started to increase within 30 seconds after the beginning of the anesthesia, but one case decreased a little at first, then increased 2 minutes later. Anesthesia reached the 3rd—the 4th plane of the 3rd stage within 5–7 minutes, and a remarkable increase of blood

flow was observed. 2–3 minutes later after the anesthesia was discontinued, the blood flow returned to the preanesthetic condition still showing a slight decrease.

In conclusion, increase of blood flow of the cerebrum and medulla oblongata was demonstrated with various anesthetics during inhalation anesthesia. In order to understand the relationship of the blood flow between the cerebrum and medulla oblongata, both curves were recorded simultaneously. (Fig. 6). Due to the different sensitivity of the two ap-

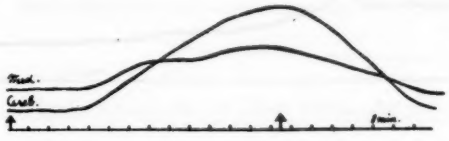


Fig. 6. Simultaneous record of the blood flow in cerebrum and medulla oblongata due to inhalation anesthesia. The first arrow shows the start of anesthesia, the second shows the stoppage of anesthesia.

paratus, an exact quantitative comparison was very difficult to carry out. However, the time relationship of the increase of the cerebrum and medulla oblongata did not show any difference concerning the beginning of increase of the blood flow, the time reaching the maximum curve, and the time returning to the normal value.

5% *Amybal* (*Intravenous anesthesia*)

4 cases received 5% Amybal 0.2–0.3 cc/kg. intravenously. The blood flow in the cerebrum and medulla oblongata decreased immedately after the injection and showed the lowest value within few minutes. The blood flow recovered afterwards, and returned to the value before the injection within 7–13 minutes excepting 1 case which died. Judging from the condition of respiration, the animal did not completely recovered from the anesthesia.

10% *Amybal* (*Intravenous anesthesia*) (Fig. 7)

4 cases had 10% Amybal 0.2–0.3 cc/kg. intravenously. Many animals showed a decrease of the blood flow in the cortex, while, in the medulla oblongata, it increased at first, then followed by a decrease. The decrease of blood flow did not recover for a long time. One case stopped its respiration immediately after the injection and died. The blood flow showed a decrease.

10% *Oltopan sodium* (*Intravenous anesthesia*) (Fig. 8)

9 cases had 10% Oltopan Sodium 0.2–0.3 cc/kg. injected intravenously. 6 cases showed an immediate reduction of blood flow in the cortex of the cerebrum and medulla oblongata. However, most of them returned close to the curve before injection within 3–5 minutes, while 2 cases showed

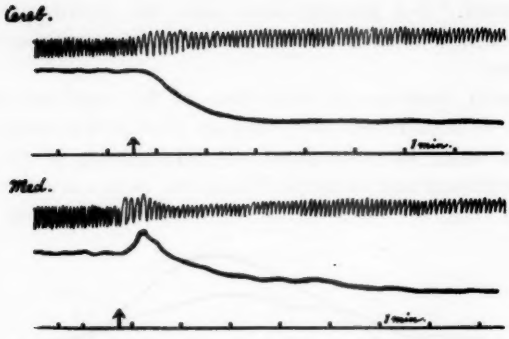


Fig. 7. Amybal. Arrow shows the intravenous injection.

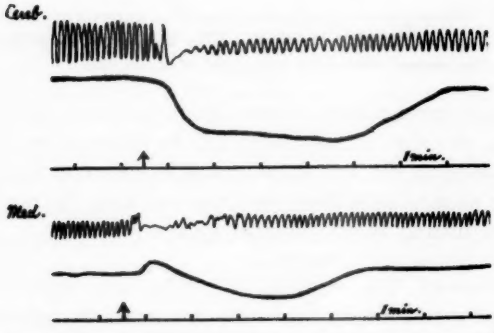


Fig. 8. Oltopan Sodium. Arrow shows the intravenous injection.

a higher curve than the beginning of the injection. In spite of the recovery of the blood flow, the animal was not completely awaken from the anesthesia which could be judged from the changes of respiration. 1 case died immediately after the injection. Simultaneous recording of the blood flow in the cerebrum and medulla oblongata during intravenous anesthesia was done in 9 cases, (Fig. 9). A slow decrease of blood flow was observed in the medulla oblongata, while a rapid decrease was shown in the cerebral cortex. Vavious changes of recovery were observed, *i.e.*, simultaneous recovery in the cerebrum and medulla oblongata; rapid recovery in the medulla oblongata in contrast to a slow recovery shown in the cerebrum.

2.0% *Morphine hydrochloride* (Fig. 10)

6 cases had intravenous injection of 2.0% morphine hydrochloride 0.1–0.2 cc/kg. Immediately after the injection, the blood flow in the cerebrum and medulla oblongata decreased. I cases showed recovery

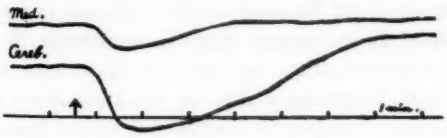


Fig. 9. Simultaneous record of the blood flow in cerebrum and medulla oblongata due to intravenous anesthesia.

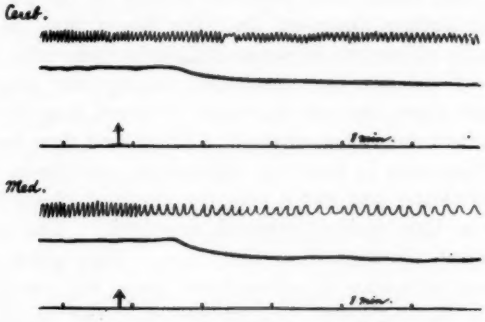


Fig. 10. Morphine hydrochloride. Arrow shows the intravenous injection.

within few minutes.

Discussion

It has been thought that ether increases the blood flow in the cerebrum. Yamakita[3] said that the blood flow increases in the excitement stage and decrease in the stage of deep anesthesia. Machida[5] stated that the increase of blood flow occurs gradually in the stage of deep anesthesia, while Iwata[6] wrote that blood flow increases during the 2nd and the 3rd stage of anesthesia. All of these experiments were studied on the changes of blood flow in the brain in toto. Schmidt studied the cortical blood flow during ether anesthesia and reported that blood flow increased gradually with slight decrease of blood pressure. Recently, Gakunaka[7] reported the increase of blood flow in the cortex of the cerebrum. No reports have appeared so far concerning the simultaneous observation of the cortex and medulla oblongata. The author has observed a very slight decrease of blood flow of the cerebrum and medulla oblongata in most of the cases during the 2nd stage of anesthesia, started to increase in the stage of surgical anesthesia and reached the maximum at the end of surgical anesthesia. Many investigators have stated that blood flow in the cerebral cortex increase during the 2nd stage, while slight decrease

of blood flow was observed in most of the authors' cases. It is thought that increased blood flow during surgical anesthesia is mainly due to the vasodilatator action of ether, and part of this change might be caused by hypercapnia and hypoxemia due to unsatisfactory respiration. In chloroform anesthesia, according to Yamakita,[3] blood flow of the cerebrum increases at first, and decrease at the later half of surgical anesthesia and continues for a rather long period; and takes considerable time to recover. Iwata[6] opined that blood flow becomes slower in the stage of surgical anesthesia. The author observed that the blood flow in the cerebral cortex and medulla oblongata increases relatively early after the beginning of anesthesia and reaches the maximum during the stage of surgical anesthesia. Some cases showed decrease of blood flow in the 3rd—the 4th plane of the 3rd stage of anesthesia. The blood flow increases earlier and the speed of increase is faster in chloroform anesthesia than in ether anesthesia. Yamakita[3] has stated that the cerebral blood flow decreases remarkably in the later half of surgical anesthesia. The author has experienced the same changes in a few cases. This phenomenon seems to be the toxious influence of chloroform upon the circulation. After the anesthesia is discontinued, remarkable decrease of the blood flow returns immediately to the preanesthetic value and some cases still show further decrease. During chloroform anesthesia, 2 cases showed weak respiration immediately after the beginning of inhalation anesthesia and decrease of blood flow was noticed, while none was observed in case of ether anesthesia.

As ethyl chloride anesthesia showed almost the same changes of blood flow as in chloroform anesthesia, the discussion is omitted here. The mechanism of increased blood flow of the brain during chloroform and ethyl chloride are thought to be the same as in ether anesthesia. Inhalation anesthetics are thought to affect the cerebral cortex at first and the medulla oblongata in the last. From the blood flow study, the circulation in the medulla oblongata changes parallel with the cerebrum. It seems that inhalation narcotics work on the vessels of cerebral cortex and medulla oblongata in the same way and the specificity of the action is mainly connected with the sensitivity of the nerve cells to these drugs.

The influence of barbiturate narcotics upon the cerebral blood flow has been reported. Tanabe said that the intravenous injection of Ravonal, Isomital, and Oltopan Sodium decrease the blood flow in the cerebrum. Decrease of blood flow in the cerebrum and medulla oblongata occurred immediately after the injection of 5% and 10% Amybal and 01% Oltopan Sodium. The blood flow returned to normal faster in case of Oltopan Sodium, while in case of Amybal, the recovery was slow, especially the injection of 10% Amybal prolonged its recovery. After the recovery

of blood flow, the influence of narcotics did not completely disappear as judged by the respiration. In other words, the recovery of blood flow was earlier than the disappearance of the influence of narcotics. The decrease of blood flow by intravenous anesthesia recovered fast in Oltopan Sodium which has only a short narcotic action, while recovery was slow in case of Amybal which has a long narcotic action. From these observations, the blood flow and narcotic influence runs parallel. The hypotensive effect of the narcotics has also some relationship.

Narcotics have been classified in cortical and subcortical drugs since Pick. However, different classifications have been proposed by many other investigators. Keeser[8] studied the distribution of barbiturates and grouped them into subcortical narcotics, but Maynert could not confirm this fact from isotope study. Kernard[9] observed the dilatation of the blood vessels in the hypothalamus and maintained that it is one of the proofs that barbiturates affect chiefly the subcortex. Recently, many reports on the effect of narcotics have been studied by EEG. Though different effects on the brain have been observed when ether and barbiturates are used, it is impossible to state that the former are cortical and the later subcortical. The author observed a remarkable increase of blood flow by cortical narcotics and its decrease by subcortical anesthetics. It is an another question whether the changes of blood flow are due to the nature of cortical and subcortical narcotics.

Morphine has been thought to influence the cortex and medulla oblongata. Slight decrease of blood flow was observed in my own experiments.

Conclusion

The influence of various narcotics upon the blood flow in cortex and medulla oblongata is studied on cats using Schmidt's thermocouple.

1. In ether anesthesia, a very slight decrease of blood flow is observed after the beginning of the anesthesia, then gradually increases and reaches the maximum at the later half of the 3rd stage of anesthesia, and after stopping the anesthesia, it returns to the preanesthetic value.

2. In chloroform, the blood flow increases rapidly in the cortex and medulla oblongata, reaches the maximum in the 3rd stage of anesthesia and in some cases, decreases in the later half of the deep anesthesia. After the anesthesia is stopped, the decrease of blood flow is remarkable and in many cases it decreases more than the preanesthetic value. Ethyl chloride follows the same pattern.

3. In inhalation anesthesia, the blood flow in the cerebrum and the medulla oblongata changes simultaneously.

4. In intravenous injection of 5% and 10% Amybal (Amytal

Sodium), the blood flow decreases in the cortex and medulla oblongata. In case of 5% Amybal, the blood flow recovers in about 12 minutes, while in case of 10% Amybal, its decrease is prolonged. In case of 10% Oltopan Sodium (Evipal Sodium), the blood flow decreases in the cortex and medulla oblongata and recovers faster than that of Amybal.

5. In case of 2% morphine hydrochloride, blood flow decreases in the cortex and medulla oblongata immediately after the injection.

6. Blood flow changes between cortical and subcortical anesthetics are observed. It is difficult to decide whether they are due to the nature of the anesthetic effect or not.

References

1) Pick, Wien. klin. Wschr., 1927, **40**, 634.
2) Schmidt, Amer. J. Physiol., 1934, **108**, 241.
3) Yamakita, Tohoku J. Exp. Med., 1922, **3**, 414.
4) Jasper, Amer. J. Physiol., 1940, **128**, 488.
5) Machita, Kyoto-Ikadaigaku-Zasshi (Jap.), 1931, **5**, 2743.
6) Iwata, Hukuoka-Ikadaigaku-Zasshi (Jap.), 1932, **25**, 1001.
7) Gakunaka, Nagasaki-Ikgakkai-Zasshi (Jap.), 1950, **25**, 89.
8) Keeser, J. Pharmacol. Exper. Therap., 1935, **53**, 137.
9) Kennard, Amer. J. Physiol., 1940, **129**, 650.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

Studies on Brain Stem

VI. The Influence of Medullar Reflex on Facial Nucleus of Mammals and Birds

By

Shigeaki Yamamoto, Shunsuke Utsushi
(山本 重亮) (宇津志 俊介)
and **Naokichi Endo**
(遠藤 直吉)

From the Anatomical Laboratory of Prof. H. Seto, Faculty of Medicine, Tohoku University, Sendai

(Received for publication, July 2, 1953)

According to Kappers'[1)2)] neurobiotaxis theory, the facial nucleus is said to be subject to two long steps of dislocation, both ontogenetically and phylogenetically, namely, 1) from the cranial position to the caudal; and 2) from the dorsal to the ventral. The second of the above dislocations is attributed to the traction of the developing pyramidal tract. Kappers[3)] also presumed that the facial nucleus in man and higher mammals is attracted toward the descending trigeminal root. In this case, his contention is that the influence of the descending trigeminal root prevails over the cortical influence exerted by the pyramidal tract, that is, for the facial nucleus, the influence of the medullar reflex is of higher importance. The dislocation of the facial nucleus toward the descending trigeminal root or lateralwards, is here evidenced by its shifting from the medial to the lateral side of the superior olive, which is taken as the standard for determining the position. We have proceeded with the experiments described hereunder for the purpose of reviewing these deductions of Kappers' theory.

Experimental

Materials

Series of transverse sections, Pal-carmine stained, of brains of mammals of various families, *i.e.*, monotremata (echidna), edentata (ant-eater), insectivora (mole), chiroptera (bat), rodentia (rabbit and japanese dancing mouse), cetacea (dike whale), ungulata (horse and goat), carnivora (cat, dog, black bear and fur seal), primates (orang-outang, macaca, gibbon, baboon, silver gibbon, siamang and platyrrhini) and man, and some birds (pigeon, japanese bush-warbler, baby crow, gull, owl and cock)

were used in the following experiments.

Findings

Upon observing the relative positions of the facial nucleus and superior olive, by the higher mammals, namely, primates and carnivora, the facial nucleus is entirely on the lateral side of the superior olive, lying abreast of it. Only by a fur-seal, the superior olive is represented by a nucleus within the facial nucleus.

In the ungulata, the pyramidal tract is only very weakly developed by a goat, but developed in an average degree by a horse. In the latter, the facial nucleus is lying entirely on the lateral side of the superior olive, touching it, while in the former, it is located slightly on the dorsolateral side of the superior olive.

According to the neurobiotaxis theory, the facial nucleus should be much more displaced to the lateral by the traction of the descending trigeminal root, by a goat than by a horse, as the influence of the pyramidal tract is so much weaker by the former than by the latter.

By a dike whale (cetacea), the pyramidal tract is very weak, not forming a bundle but scattered in groups of fibres, but the facial nucleus is entirely on the lateral side of the superior olive.

Rabbits and japanese dancing mice belong to a same genus of the rodentia family, but the pyramidal tract is much better developed in the latter than in the former. The facial nucleus, however, is in an almost dorsal position, only slightly tending to the lateral, against the superior olive in both of these animals, showing no noticeable difference in location. This finding is again not in agreement with the neurobiotaxis theory.

By a bat (chiroptera) and a mole (insectivora), the pyramidal tract is weak, but the facial nucleus is almost on the dorsal against the superior olive, with a slight leaning to the medial.

In the brain of an ant-eater (edentata), the pyramidal tract is developed normally and the facial nucleus is positioned almost dorsally to the superior olive. The relative position of the facial nucleus is nearly the same by a echidna (monotremata), while the development of the pyramidal tract is extremely poor.

In the brain of birds, the facial nucleus is situated almost dorsally (only a little to the lateral) by a pigeon, nearly dorsally by a bush-warbler, dorsomedially by a baby crow, medially by a gull, dorsolaterally by an owl, and medially by a cock.

Discussion

According to the neurobiotaxis theory by Kappers, the facial nucleus

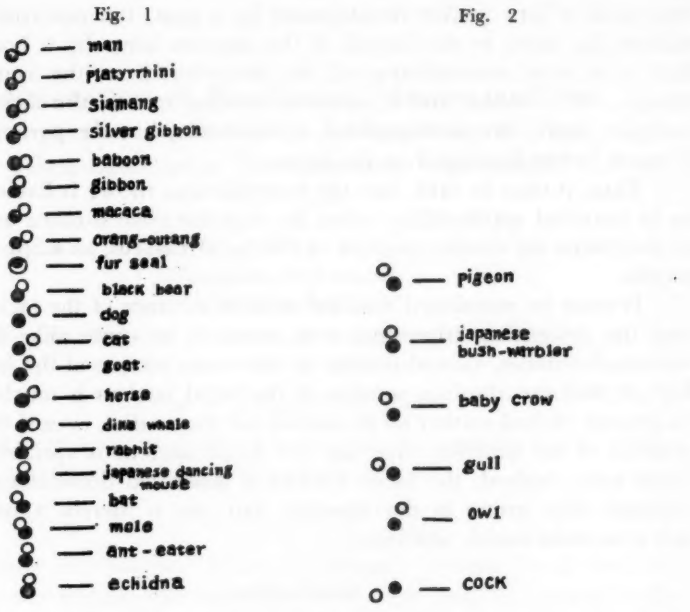


Fig. 1. Topographic relations of facial nucleus (white marks) and superior olive (black marks) at their caudal ends in various mammals.

Fig. 2. Topographic relations of facial nucleus (white marks) and superior olive (black marks) at their caudal ends in certain birds.

is shifted toward the pyramidal tract, and then, toward the descending trigeminal root, the strongest of the medullar reflexes. Our observations have revealed rather numerous points in divergence from such assumptions.

Firstly, by birds, who have no pyramidal tract, the influence of the descending trigeminal root must be relatively stronger than by mammals, but in our specimens, the facial nucleus was seen on the dorsal or medial side of the superior olive in every case except that of an owl. On the other hand, in mammals, especially in the higher ones, the facial nucleus is on the lateral side of the superior olive, as Kappers would have it. But if the facial nucleus be attracted by the descending trigeminal root, it follows that its traction would be relatively greater in the cases when the development of the pyramidal tract is weaker, but in truth, as described above, often the opposite phenomenon is observed. In the lower genera of the mammals, the facial nucleus, generally speaking, is found lying close to the dorsal, in some cases lightly leaning to the medial side of the superior olive. Again, in comparing the brains of a horse and a goat, who both belong to the same family of ungulates, the pyramidal

tract is in a very weaker development by a goat, but nevertheless facial nucleus far more to the lateral of the superior olive by a horse. This fact is in utter contradiction of the deduction from the neurobiotaxis theory. By a rabbit and a japanese dancing mouse, the facial nucleus occupies nearly the same position in the brain, but the pyramidal tract is much better developed in the latter.

Thus, it may be said, that the neurobiotaxis theory is liable to doubt in its practical applicability, when the superior olive is taken as the basis to determine the relative position of the facial nucleus, as Kappers recommends.

It must be mentioned that the relative distance of the facial nucleus and the descending trigeminal root seems to be quite alike by all the examined animals, viewed relative to the entire section of the brain stem. Isn't it that the absolute position of the facial nucleus is much the same in general, shifted neither to the lateral nor the medial, even if the relative position of the superior olive and the facial nucleus is variable? But in many cases, indeed, the facial nucleus is dislocated dorsolaterally as the superior olive grows in development, but this is merely a mechanical, not a neurobiotaxial, shifting.

Conclusion

Upon examination of the influence of the medullar reflexes on the position of the facial nucleus, we found that the facial nucleus is generally not located on the lateral side of the superior olive by birds, who lack pyramidal tract, whereas by the higher mammals, who have their pyramidal tracts well developed, it is generally located thus. In cases where different species belonging to the same family show strong contrast in the development of the pyramidal tract, the same discrepancy may be observed, or otherwise the position of the facial nucleus is quite analogous, in spite of the difference in the development of the pyramidal tract. Even admitting the traction of the medullar reflexes, according to the neurobiotaxis theory, we must naturally expect the contrary to happen, in considering the influence of the pyramidal tract. From these views, we feel justified in doubting the adequacy of Kappers' neurobiotaxis theory.

References

1) Kappers, Neurol. Zentralbl., 1907, **26**, 834.
2) Kappers & Vogt, Neurol. Zentralbl., 1908, **27**, 958.
3) Kappers, Comparative anatomy of nervous system of vertebrates, Vol. 1, Macmillan, New York, 1936.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

Fractionation of Pig Gastric Mucosa and the Properties particularly Antigenicity of the High-molecular Components separated

First Paper

By

Kohsiroh Tokutomi
(徳富 紅紫郎)

From the Medico-chemical Institute, Tohoku University, Sendai;
Director: Prof. H. Masamune

(Received for publication, July 3, 1953)

The present account deals with the isolation procedure and several properties, mostly chemical, of the products.

Experimental

Preliminary fractionation. Fresh pig stomach mucosa, freed mechanically from the mucus and muscular layer, was roughly washed under running water, wiped and ground. 250 g. of the pulp thus obtained (from two stomachs) were shaken with 10 volumes of physiological saline (10 cc. of toluene were added as a preservative) on a machine for 3 days. Of the three layers after centrifugation, the upper was discarded, the middle (solution) set aside and the lower (tissue residue) subjected twice more to a similar extraction. The successive solutions were treated separately, as will be described in some detail regarding the run with the first solution:—the slightly turbid fluid (2.2 *l.*) was dialyzed for 3 days against running water and the appearing sediment centrifuged off. The brownish, translucent supernatant (2.5 *l.*) was acidified to pH 4.6 with 8 cc. of 30% acetic acid and stood in an ice chamber overnight. The voluminous flocculent precipitate was separated, and washed with dilute acetic acid of pH 4.6, alcohol and ether and dried—a brownish powder, 5 g. The clear yellowish mother fluid and acetic acid-washing were combined and distilled in vacuo to about 20 cc. and the deposit after addition of 5 volumes of abs. alcohol was washed with alcohol and ether and dried as above. A yellow powder given here amounted to 5.2 g. The total yield of the acid-insoluble (**I**) and -soluble (**II**) fractions of all the tissue extracts were 5.7 and 5.8 g. respectively.

Rough subdivision of the acid-insoluble fraction. 5.6 g. of this fraction were agitated with 100 cc. of water, adding drop by drop of

1% NaOH up to a pH of 6.6. A small insoluble part was rejected. The brown solution was precipitated by shifting the pH to 4.6 with acetic acid, separated and washed with the dilute acetic acid of pH 4.6. Redissolution and reprecipitation was carried out twice more.

From the mother fluids and acetic acid-washings were recovered 1.4 g. of a yellowish powder by distillation in vacuo and precipitation with alcohol.

Next, the repeatedly reprecipitated acid-insoluble was subjected to Kerr procedure:—After dissolution of it in 100 cc. of water similar to above (pH of the solution 6.6), 17.5 g. of NaCl were added to 3 *M*, and the solution was placed in an ice chamber for 3 days. A precipitate began to appear in 30 minutes. The deposit was centrifuged (Standing of the supernatant gave no deposit) and washed with 3 *M* NaCl, then it was dialyzed in 50 cc. of water (against running water for 3 days), distilled in vacuo to about 5 cc. and precipitated with 25 cc. of abs. alcohol. A brownish powder weighing 1.06 g. when dry was given—**Crude Protein I.** Biuret strongly positive. Molisch positive. Phosphorus was detected though minute. To the mother fluid (containing 3 *M* NaCl) and washing united (140 cc. altogether) were added 5% HCl in drops to pH 4.2. On standing the fluid in an ice chamber overnight came out a bulky flocculent precipitate again. This was centrifuged and washed with 3 *M* NaCl acidified to pH 4.2 with HCl, then it was freed from NaCl by dissolution in 50 cc. of water, dialysis, condensation and precipitation with alcohol. The product dried (a brownish powder) weighed 1.8 g.—**Crude Protein II.** The qualitative tests resulted as in Crude Protein I.

The mother fluid of Crude Protein II and washing (200 cc. in all) were poured into 2 volumes of abs. alcohol under agitation. After 1 hour, the deposit (A large amount of NaCl also was carried down together) was centrifuged, washed at first exhaustively with methanol to remove NaCl and then with alcohol and with ether and dried—A yellowish powder, 520 mg. (**Subd. III**). Molisch, Neuberg-Saneyoshi and Bial-Mejbaum (for pentose), positive. Biuret slightly positive, Dische (for desoxypentose) negative. Phosphorus and purine detected. Namely the substance here was a mixture of PNA and a polyuronic acid or polyuronic acids, contaminated with a little protein.

The mother fluid and methanolic washing (600 cc. altogether) were vacuum-distilled to about 100 cc. to expel off the alcohols, dialyzed for 5 days to eliminate NaCl and HCl, and further condensed to about 5 cc. The thick solution was precipitated with 5 volumes of abs. alcohol, washed with ether and dried—a white powder weighing 115 mg. Biuret, Molisch and Neuberg-Saneyoshi positive. Phosphorus was detected in trace.

Purification of Protein I and its properties. 1 g. of Crude Protein I was taken up in 50 cc. of water (pH of the solution 6.4), an insoluble part removed and NaCl added to the solution to 3 *M* (8.8 g. were used), followed by standing in an ice chamber for 1 day. The flocculent precipitate was centrifuged, washed with 3 *M* NaCl twice at first and then with a dilute acetic acid of pH 4.6 several times to eliminate NaCl. The substance was again taken up in 50 cc. of water, adjusting the pH to 6.4 with drops of 1% NaOH, to repeat the process. Redissolution and reprecipitation was processed furtheron to get rid of Molisch-giving impurities (repeated 7 times) and the final precipitate, washed thoroughly with the dilute acetic acid, was washed with alcohol and ether and dried to constant in vacuo over $CaCl_2$. 240 mg. of a light-yellow powder were yielded—**Protein I.**

It was strongly biuret-positive. Molisch almost negative. Neuberg-Saneyoshi and Goldschmiedt negative. Phosphorus was not detected. Electrophoresis of it at pH 7.8 (in a Tiselius apparatus) demonstrated a single boundary (Fig. 1). It dissolved in water, but on acidification,

Fig. 1. An electrophoretic pattern of a 1% solution of Protein I in phosphate buffer, pH 7.8, $\Gamma=0.29$; temperature 19.5°C; current 10 ma; exposure 60 minutes. Mobility: 10.2 $cm^2 \cdot volt^{-1} \cdot sec^{-1} \cdot 10^5$ Ascending limb to the left.

precipitated at pH range from 4.6 to 2.0 and dissolved again at a lower pH. It was also soluble in 99% acetic acid, but not in 1% trichloroacetic acid, the saturated $(NH_4)_2SO_4$ and 3 *M* or more concentrated NaCl. Precipitated by cupric chloride, lead acetate and barium acetate. N (micro Kjeldahl) 14.8%, ash (micro Pregl without the use of H_2SO_4) 3.9%. Specific refraction (19.2 mg. of the substance were dissolved in 2 cc. of 0.01 *N* NaOH. pH of the solution, 8.0. Examined in an Abbe refractometer and an Ostwald viscosimeter. Temperature of test 17.5°C).

$$\frac{(1.33518)^2-1}{(1.33518)^2+2}\times\frac{1}{1.0036}\times\frac{100}{0.96}-\frac{(1.33366)^2-1}{(1.33366)^2+2}\times\frac{1}{1.005}\times\frac{100-0.96}{0.96}=0.236^\circ$$

Rotation (1 cc. of the solution above was diluted twice with water).

$$[\alpha]_D=\frac{-0.3374\times 1}{0.0048\times 1}=-73.7^\circ$$

Purification of Protein II and its properties. 1.7 g. of Crude Protein II

were dissolved in 50 cc. of water, drop by drop of 1% NaOH added to pH 6.4 and an insoluble part filtered off. After addition of 8.8 g. of NaCl, the filtrate was placed in an ice chamber. And, after 2 days the precipitate was discarded, and the mother fluid was acidified to pH 4.2 with 1% HCl and kept again at 0°C overnight. The precipitate hereby was centrifuged and washed at first with 3 *M* NaCl, acidified to pH 4.2 with HCl, and afterwards with a dilute acetic acid solution of pH 4.6 as above. The whole procedure was repeated 5 more times, and finally the substance was washed with alcohol and ether and dried to a constant weight in vacuo over $CaCl_2$. The product (a yellowish powder)—**Protein II**—amounted to 310 mg.

It gave strong biuret reaction. Molisch, Neuberg-Saneyoshi and Goldschmiedt negative. Phosphorus was absent. A pattern after electrophoresis at pH 7.8 (Fig. 2) showed a single symmetric boundary. N

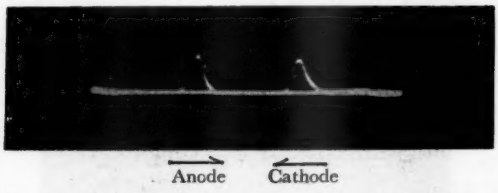


Fig. 2. An electrophoretic pattern of a 1% solution of Protein II in phosphate buffer, pH 7.8, I=0.29; temperature 18.5°C; current 10 ma; exposure 60 minutes. Mobility: 11.1 $cm^2 \cdot volt^{-1} \cdot sec^{-1} \cdot 10^5$. Ascending limb to the left.

15.0%, ash 0.6%. It dissolved in water. On acidification of the solution with HCl, it began to precipitate at pH 4.6 to again dissolve at pH<2.0. It was carried down by $CuCl_2$ and lead acetate, but not by barium acetate and barium chloride. It was also soluble in 3 *M* and more concentrated NaCl and 99% acetic acid, but insoluble in 1% trichloroacetic acid and the saturated $(NH_4)_2SO_4$. Specific refraction (18.7 mg. of the substance were dissolved in 2 cc. of 0.01 *N* NaOH. pH of the solution 7.8. Temperature of test 17°C).

$$\frac{(1.33509)^2-1}{(1.33509)^2+2}\times\frac{1}{1.0033}\times\frac{100}{0.94}-\frac{(1.33366)^2-1}{(1.33366)^2+2}\times\frac{1}{1.0005}\times\frac{100-0.94}{0.94}=0.249^\circ$$

Rotation (1 cc. of the solution above was diluted with 1 cc. of water).

$$[\alpha]_D^{25}=\frac{-0.3374\times 1}{0.0044\times 1}=-72.3^\circ$$

Separation of pentosenucleic acid and a mixture of hexuronic acid-containing polysaccharides. Subd. III of the acid-insoluble fraction above was liberated first from proteins as follows. 500 mg. of it were agitated in 30 cc. of water, under addition of 1% NaOH till persistent pH 6.2, and an insoluble

part centrifuged off. The clear solution was freed from the part precipitable by NaCl of 3 molarities at pH's 6.2 and 4.2 as described in the preceding section and 2 volumes of abs. alcohol added. The deposit was washed with methanol exhaustively then with alcohol and ether roughly and dried. The operations were processed again and again until the biuret test resulted in negative (7 times altogether). 210 mg. was the yellowish powder given. Molisch, Neuberg-Saneyoshi and Bial-Mejbaum positive. Dische negative. Phosphorus and purine detected. In the next, 200 mg. of this product were dissolved in 10 cc. water by the aid of 0.5% NaOH (pH of the solution 6.2), and after addition of 20% $CuCl_2$ in drops to pH 4.2, placed in an ice chamber for 6 hours. The precipitate here was centrifuged, washed with 0.5% $CuCl_2$ (The supernatant and washings were set aside to separate the hexuronic acid-containing polysaccharides. See below) and suspended in 5 cc. of water followed by passing H_2S. CuS was rejected (by centrifugation and filtration) and to the clear filtrate were added 5 volumes of abs. alcohol. Finally the precipitate, centrifuged and washed with ether, was taken up in 10 cc. of water and after centrifuging off of a trace of an insoluble part, 0.5% HCl was added to pH 2.0. The flocculent precipitate occurring hereby was separated, washed with a dilute HCl of pH 2.0, abs. alcohol, to which a little concentrated HCl had been added to pH 2.0, plain abs. alcohol and ether in sequence and dried to constant. 95 mg. of a white powder (**PNA**) were given.

The centrifuged supernatant and washings set aside above (24 cc. in all) were passed through a thin kaolin layer to remove floating particles, and the filtrate was bubbled with H_2S. The filtrate from CuS and washing (in total 40 cc.)were condensed by vacuum distillation to about 5 cc., precipitated with 5 volumes of abs. alcohol and after washing with ether, dried to constant. A white powder weighing 32 mg. was given.—**Polyuronic Acids.**

Properties of the nucleic acid and the hexuronic acid-containing polysaccharides. Products after several runs were united for the examination. 1) PNA. Biuret, Neuberg-Saneyoshi and Dische negative. Molisch and Bial-Mejbaum (for pentose) positive. Phosphorus and purine detected. It dissolved in water, when NaOH was added to pH 4.2–4.4, and was precipitated from a solution of pH 5.6 by $CuCl_2$, lead acetate, barium acetate and baryta. Insoluble was it in 99% acetic acid and the saturated $(NH_4)_2SO_4$. Electrophoresis at pH 7.8 gave a pattern demonstrating one symmetric boundary (Fig. 3). N (micro Kjeldahl) 14.8%; P (a modified Plimmer method) 7.5%; ribose (Masamune and Ogawa[2]) 33.8%; ash 6.8%. N:P=2.0:1.0. Specific refraction (22.13 mg. of the substance were dissolved in 2 cc. of 0.01 *N* NaOH. pH of the solution

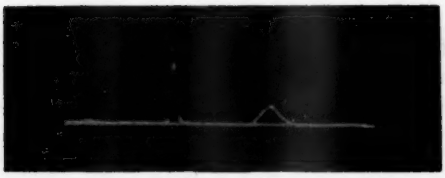


Fig. 3. An electrophoretic pattern of a 1% solution of PNA in phosphate buffer, pH 7.8, I=0.29; temperature 12.5°C; current 10 ma; exposure 30 minutes. Mobility: 19.8 $cm^2 \cdot volt^{-1} \cdot sec^{-1} \cdot 10^5$ Ascending limb to the left.

7.2. Temperature of test 17.5°C).

$$\frac{(1.33515)^2-1}{(1.33515)^2+2}\times\frac{1}{1.0068}\times\frac{100}{1.11}-\frac{(1.33366)^2-1}{(1.33366)^2+2}\times\frac{1}{1.0005}\times\frac{100-1.11}{1.11}=0.269^\circ$$

Rotation (The solution above was employed).

$$[\alpha]_D^{17.5}=\frac{1.2574\times 1}{0.0111\times 1}=+113.1^\circ$$

2) The hexuronic acid-containing polysaccharides. a) Test-tube tests. Biuret and Molisch, negative. Phosphorus absent. Goldschmiedt, Neuberg-Saneyoshi and Bial-Mejbaum (for pentose) positive. Hydrolysable sulfur detected. b) Paper chromatography of sugar components. 12 mg. of the substance were heated with 2 cc. of 1 *N* H_2SO_4, as sealed in an ampoule, at 100°C for 12 hours. After dilution twice with water, the hydrolysate was nearly neutralized (to pH 3.6) with baryta. The precipitate was centrifuged and washed with water and the supernatant and washing were distilled in vacuo to 0.1 cc. The centrifuged supernatant was adjusted to pH 2.8 with a dilute H_2SO_4 solution and 0.03 cc. portions were sampled for descending irrigation. Temperature of test 23–26°C. Solvent, *n*-butanol-pyridine-water (5:3:2). Indicator, aniline-phthalic acid reagent of Partridge. As Fig. 4 demonstrates, it contained galacturonic and glucuronic acid and glucosamine. c) Quantitative analysis. N 3.04%. Hexosamine 30.46%. Hydrolysable sulfur (gravimetrically measured as $BaSO_4$) 5.2%. Hexuronic acids: orcinol method, 30.5% as galacturonic acid or 28.5% as glucuronic acid; indole method, 32.2% as galacturonic acid or 65.8% as glucuronic acid. The values of hexuronic acids as galacturonic acid, obtained by the two color reactions, coincide with each other much better than those as glucuronic acid. Moreover they nearly correspond to 1 equivalent of hexosamine. Thus it appears that galacturonic acid preponderated. (The equivalent ratios of N:hexosamine:hexuronic acids as galacturonic acid (average): hydrolysable S=1.27:1.0:0.95:0.95.)

Separation of the group carbohydrate (*polypeptide-polysaccharide complex*). 5.8

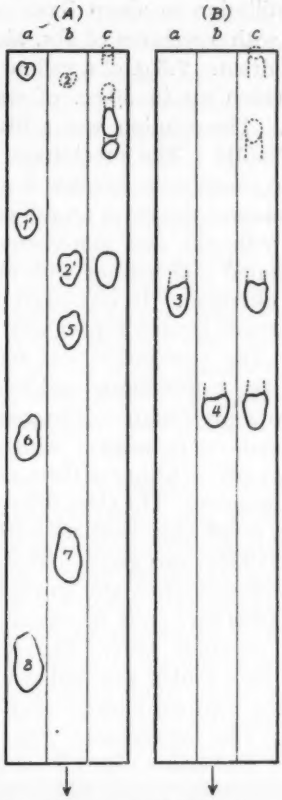


Fig. 4. *n*-Butanol-pyridine-water (5:3:2) chromatograms of a hydrolysate of Polyuronic Acids, obtained in the descending way. Temperature of test, 23–26°C. Indicator, aniline-phthalic acid of Partridge.

(A): irrigated for 24 hrs. (B): irrigated for 96 hrs. a and b: reference runs. c: Polyuronic Acids. 1 and 1′: chondrosamine sulfate, 2 and 2′: glucosamine sulfate, 3: galacturonic acid, 4: glucuronic acid, 5: galactose, 6: mannose, 7: L-fucose, 8: rhamnose.

g. of the acid-soluble fraction (Fr. II) of the tissue extract (See the section "Preliminary fractionation") were dissolved in 100 cc. of water by the aid of 1% NaOH (pH of the solution 7.0). After centrifuging off of a small insoluble part (96 mg.), the solution (brownish, slightly turbid and somewhat viscid) was acidified to pH 4.6 with drops of 30% acetic acid and stood overnight in an ice chamber. The massive flocculent precipitate was centrifuged, washed with a dilute acetic acid of the same pH and rejected. The supernatant and washing were dialyzed for 3 days,

followed by vacuum distillation to about 8 cc. And the ropy thick solution were precipitated with 5 volumes of abs. alcohol and after washing with alcohol and ether, dried. 2.6 g. of a yellow powder were recovered. Then 2.5 g. of it were taken up in 50 cc. of water (A small part undissolved was discarded. The solution was a little viscid) and alkalized to pH 9.0 with 1% NaOH. The precipitate, which was centrifuged off, was chiefly inorganic phosphates, because it gave negative biuret and Molisch reaction and remained mostly as an ash containing P on ignition. The supernatant (clear yellowish and non-viscous) was condensed after 2 days' dialysis, precipitated and washed with alcohol and dried. 1.1 g. of a white powder were obtained. It was taken up in 30 cc. of water (pH of the solution 6.4. A small insoluble part was taken off) and 1% HCl added to a pH of 4.2. The precipitate was separated off and to the mother fluid was added the hydrochloric acid solution further to pH 2.0, which again gave rise to a precipitate. The precipitates at pH's 4.2 and 2.0 (both small in amount) were washed with very dilute hydrochloric acid solutions of respective pH's. Either of them contained phosphorus and were Molisch and biuret positive. The clear solution was precipitated with alcohol (5 volumes) and dried after washing with alcohol and ether. A white powder weighing 910 mg. was given. This gave the strong Molisch reaction, but was still biuret-positive and proved to contain phosphorus.

Next, the balance (900 mg.) was dissolved in 20 cc. of water and liberated from a small insoluble part. The solution was viscous and slightly acid (pH 6.2). 20% $CuCl_2$ was added in drops (0.5 cc. in total) to pH 4.4, whereupon the solution became slightly turbid. Stood overnight and centrifuged. The supernatant was dialyzed (for 3 days), distilled in vacuo to 5 cc. and poured into 5 volumes of abs. alcohol containing a little concentrated HCl (pH 2.0). The deposit was washed more than ten times with the HCl-containing alcohol to remove Cu, then with plain abs. alcohol thoroughly to remove HCl and with ether. When dried, the substance (a white powder) weighed 624 mg. It was free from phosphorus, but gave slightly positive biuret beside Molisch.

600 g. of it were dissolved in 40 cc. of water. To the clear, viscous solution after rejecting a trace of an insoluble part (pH 4.8) was added 10% lead acetate in small portions just to carry down all the precipitable (2.5 cc. were required). The centrifugate was washed with 1% lead acetate, followed by centrifugation. And the mother fluid and washing (31 cc. in total) were combined, bubbled with H_2S and filtered with suction through a thin layer of kaolin (placed on a filter paper). The non-colored transparent filtrate was distilled in vacuo to about 7 cc. (pH of the condensate 3.0) and precipitated with alcohol and anhydrous sodium acetate, then the deposit was washed with alcohol and ether

and dried. Yield, 219 mg. This gave strong Molisch reaction but no biuret. Since, however, it contained much of inorganic sulfates, 200 mg. of it were dissolved in 5 cc. of water, and 1% $BaCl_2$ was added drop by drop, until no more precipitate occurred. To the centrifuged supernatant were added 5 volumes of abs. alcohol and a little anhydrous sodium acetate. The deposit here was washed exhaustively with abs. alcohol and then with ether and dried constant in vacuo over $CaCl_2$. 190 mg. was the white powder obtained—**Group Carbohydrate.**

The lead acetate-precipitate was suspended in water, treated with H_2S and filtered. The substance precipitated from the filtrate with alcohol (6 mg.) was biuret-positive but Molisch-negative. P present. On the other hand, the bluk on the funnel was washed with hot water and a dilute NaOH (pH 8.0), and the filtrates distilled to 5 cc. under diminished pressure and precipitated with alcohol. 6 mg. of a white powder were given. This was biuret negative and contained no phosphorus, but gave positive Molisch and Neuberg-Saneyoshi reaction.

Properties of the Group Carbohydrate. a) Test-tube tests. It gave strong Molisch, but neither biuret nor Neuberg-Saneyoshi nor Bial-Mejbaum. Phosphorus and inorganic sulfates absent. Hydrolysable sulfur present. Whether or not preliminarily heated with alkali, it developed a red color when treated with Ehrlich reagent. b) Solubility. It was soluble in water, and unprecipitable from the solution by trichloroacetic acid, $CuCl_2$, lead acetate, barium acetate and $(NH_4)_2SO_4$. In 99% acetic acid was it scarcely soluble. c) Homogeneity. After electrophoresis of it at pH 7.8, a single symmetric boundary was demonstrated (Fig. 5). d) Paper partition chromatography. i) Sugars. 20 mg. of

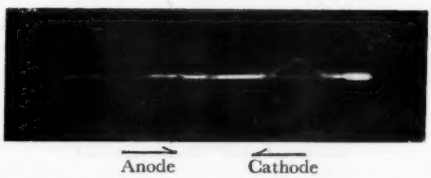


Fig. 5. An electrophoretic pattern of a 1% solution of Group Carbohydrate in phosphate buffer, pH 7.8, I=0.29; temperature 18°C; current 10 ma; exposure 150 minutes. Mobility: 1.5 $cm^2 \cdot volt^{-1} \cdot sec^{-1} \cdot 10^5$. Ascending limb to the left.

the substance were sealed with 4 cc. of 1 *N* H_2SO_4 in an ampoule and heated in a boiling water-bath for 6 hours. The hydrolysate was diluted with an equal volume of water and nearly neutralized (to pH 5.8) with baryta, and the centrifuged supernatant and washing were distilled together in vacuo to 0.1 cc., of which 0.03 cc. portions were sampled. The

chromatograms (Fig. 6) showed spots corresponding to glucosamine, chondrosamine, galactose, glucose (a weak spot) (Comp. w. the description regarding quantitative analyses below) and L-fucose. ii) Amino acids. 30 mg. of the substance were hydrolyzed with 3 cc. of 6 *N* HCl for 40 hours but otherwise similar to above. The hydrolysate was freed from

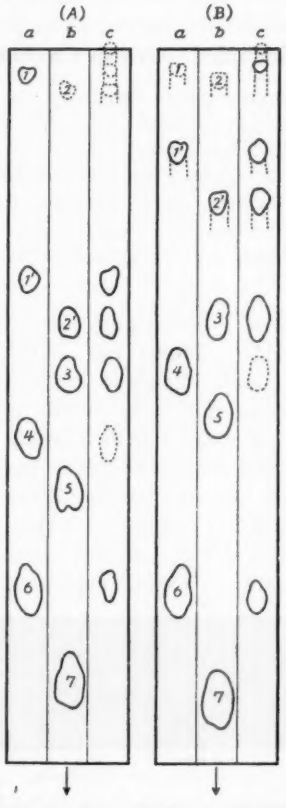


Fig. 6. Sugar chromatograms of Group Carbohydrate, obtained by descending irrigation for 24 hrs. with (A) *n*-butanol-pyridine-water (5:3:2) and (B) *n*-butylacetate-acetic acid-ethanol-water (3:2:1:1) respectively. Temperature of test 24–26°C.

a and b: reference runs; c: Group Carbohydrate. 1 and 1′: chondrosamine sulfate, 2 and 2′: glucosamine sulfate, 3: galactose, 4: glucose, 5: mannose, 6: L-fucose, 7: rhamnose.

HCl as far as possible by repeated evaporations, using water to redissolve the residue in the second and following times. The final residue was

taken up in 0.1 cc. of water and filtered and a 0.03 cc. portion was sampled. The chromatogram (Fig. 7) revealed the presence of glycine, alanine, serine, methionine and/or valine, leucine and/or isoleucine, arginine, aspartic and glutamic acid, tyrosine, histidine and proline. e) Quantitative analysis. N 3.7%, hexosamine 36.9%, galactose (Masamune and

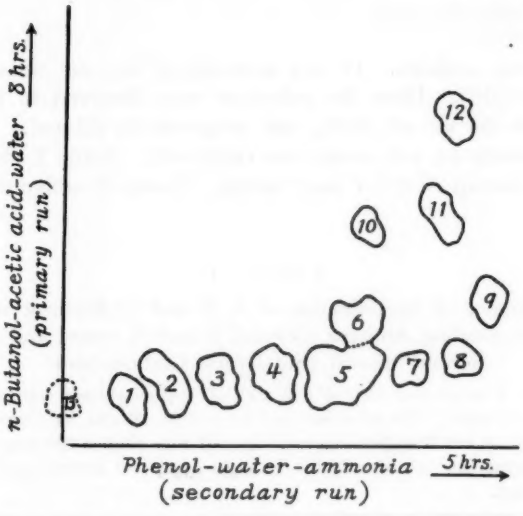


Fig. 7. Amino acid chromatogram of the Group Carbohydrate, obtained by ascending irrigation primarily with *n*-butanol-acetic acid-water (4:1:5) and secondarily with phenol (80%) in atmosphere of NH_3. Indicator, ninhydrin. Temperature of test 24°C.

1: aspartic acid, 2: glutamic acid, 3: serine, 4: glycine, 5: hexosamine, 6: alanine, 7: histidine, 8: arginine, 9: proline, 10: tyrosine, 11: valine and/or methionine, 12: leucine and/or isoleucine, 13: a peptide.

Ogawa[1]) 32.9%, L-fucose (Masamune and Ogawa[3]) 8.1%, hydrolysable sulfur (determined gravimetrically as $BaSO_4$) 0.94%, ash (Pregl method without use of H_2SO_4) 8.4%. Equivalent ratios of N:hexosamine : galactose : L-fucose : hydrolysable sulfur=1.28:1.0 : 0.89:0.24:0.14. By the hexose determination method of Masamune and Ogawa, no absorption due either to glucose or to mannose was found at the wave length of 495 mμ. f) Relative viscosity (19.4 mg. of the substance were dissolved in 2 cc. of water for measurement in an Ostwald viscosimeter. Temperature of test 20°C).

Time of outflow water, 40.4 sec.
solution, 48.4 sec.

Specific gravity (Ostwald viscosimeter): $d_4^{20}=\frac{1.00925\times 0.99823}{1.00435}=1.0026$

$\eta_{rel}=\frac{1.0026\times 48.4}{0.9982\times 40.4}=1.203$

g) Optical rotation in water (11.8 mg. of the substance were dissolved in 1 cc. of water).

$[\alpha]_D^{20}=\frac{-0.1643\times 1}{0.0118\times 1}=-13.9°$

h) Blood group activity. It was determined by our usual procedure (Cf. e.g. Oh-Uti[4]). Here the substance was dissolved in physiological saline without the use of alkali, and progressively diluted. To measure Group O activity an eel serum was employed. Table I shows that it was strongly Group A active and, besides, Group O active though much less.

TABLE I

Occurrence of Agglutination of A, B and O Erythrocytes by Corresponding Antisera (Normal B and A serum and Eel Serum) treated with Group Carbohydrate

B and A serum had titres of 640 and 160 respectively and were diluted 40 and 10 times. The eel serum had titres 3200, 20 and 160 respectively toward O, A and B erythrocytes and was used after dilution 200 times. −: no agglutination, ±: faint aggl., +: slight aggl., ++: marked aggl., ###: strong aggl.

Group of human red cells	Dilution of Group Carbohydrate (1 :)											Control*
	10^2	5×10^2	10^3	5×10^3	10^4	5×10^4	10^5	5×10^5	10^6	2×10^6	5×10^6	
A	−	−	−	−	−	−	−	−	−	+	+	###
B	+	++	###	###	###	###	###	###	###	###	###	###
O	−	−	−	−	±	+	+	+	++	++	++	++

* The sera were not treated with the Group Carbohydrate in advance.

SUMMARY

1. The extract by the aid of physiological saline of pig stomach mucosa was fractioned and two proteins, a pentosenucleic acid and a group carbohydrate were isolated in electrophoretically homogeneous state. Besides, preparations of hexuronic acid-containing polysaccharides (polyuronic acids) were obtained.

2. The properties of the substances yielded were examined.

Through the Grant Committee for Scientific Researches was given a grant from the Education Department in aid to us. H. Masamune.

References

1) Masamune & Ogawa, Tohoku J. Exp. Med., in press.
2) Masamune & Ogawa, *ibid.*, in press.
3) Masamune & Ogawa, *ibid.*, in press.
4) Oh-Uti, *ibid.*, 1949, **51**, 297.

The Tohoku Journal of Experimental Medicine, Vol. 59, Nos. 1-2, 1953

Fractionation of Pig Gastric Mucosa and the Properties particularly Antigenicity of the High-molecular Components separated

Second Paper*

By

Kohsiroh Tokutomi
(徳富 紅紫郎)

From the Medico-chemical Institute (Director: Prof. H. Masamune) and the Forensic Medical Institute (Director: Prof. T. Murakami), Tohoku University, Sendai

(Received for publication, July 3, 1953)

Rabbit antiserum of gastric mucosa, liver and kidney cortex of pig and gastric mucosa of dog (physiological saline extracts) were prepared and the serological reactivity of the high molecular components of pig stomach mucosa, which had been previously reported[1], with the sera, as such and after absorption with pig serum and one of the extracts of the tissues above and mucosa of portions of the pig digestive tract other than stomach, was examined. The results indicated that the pentose-nucleic acid of stomach mucosa is extremely organ-specific, if not strictly, contrary to the other components of the same tissue, which, though, are not devoid of somewhat characteristic feature.

Experimental

Methods

I. Immune sera

1. Immunization method in general

a) A tissue or an organ mechanically freed from adjacent tissues (mucus, membranes, blood vessels or others) was washed for a short time under running water and wiped with filter paper. It was then minced, ground, and shaken with 10 volumes of physiological saline (Some toluene was added as a preservative) for 3 days. The residue was rejected by percolation and subsequent filtration in an ice chamber. The filtrate was dialyzed in a cellophane bag against running water for 3–5 days, and after filtering off the appearing sediment, the clear filtrate

* The 2nd report of Masamune and coworkers' "Nucleic Acids."

was preserved in a brown bottle with 1/10–1/20 volume of 5% phenol after addition of NaCl to 0.85% in the mixture.

b) To a male rabbit of about 2.5 kg. were injected intravenously 3–7 cc. of an antigen solution, prepared as above, every 3 days. The injection was repeated 5–8 times and after 4–7 days from the last injection (The antigen titre was found mostly 500 (maximum dilution in terms of times of the immunogen solution where precipitin reaction occurred) and the antibody titre 8–16 (the corresponding dilution of the antiserum)), the animal was deprived of food for 1 day to shed blood from carotid artery. The serum was separated in a usual manner after standing the blood in an incubator of 37°C for 30 minutes and in an ice chamber overnight. It was inactivated by exposing to 56°C for 30 minutes and after addition of 1/9 volume of 5% phenol containing 0.85% NaCl, preserved in an ice chamber as sealed in a brown ampoule.

2. Anti-pig gastric mucosa-sera

The immunogen solution. The mucosa was teared off from the muscle layer and after freeing from the mucus, empolyed as a material. Of the solution (prior to addition of phenol), 100 cc. were distilled in vacuo to a syrup and dehydrated with alcohol. The insoluble weighed 154 mg., when dried in vacuo over $CaCl_2$.

Six antisera prepared were of antigen titre of 512 and of antibody titre of 16 or 32.

3. Anti-pig liver-sera

An immunogen solution prior to addition of phenol, which had been prepared as above, was found to contain 0.19% alcohol-insoluble components, as measured as in *2*. The immune serum had antigen and antibody titre of 512 and 8 respectively—anti-pig liver-serum I.

Here another antiserum was also prepared, by employing an antigen solution obtained without dialysis. This solution was markedly biuret- and Molisch-positive, fairly Bial-Mejbaum- and Dische-positive and contained phosphorus and purine. The alcohol-insoluble components of it amounted to 0.32%. The immune serum had antigen and antibody titre of 2048 and 32 respectively—Anti-pig liver-serum II.

4. Anti-pig kidney cortex-sera

The immunogen solution obtained showed similar results in test-tube tests as the solution used for obtaining Anti-pig liver-serum II and contained 0.23% alcohol-insoluble components. Either of two antisera given had an antigen titre of 520 and an antibody titre of 32.

5. Anti-canine stomach mucosa-sera

The canine stomach mucosa, wherefrom the mucus had been scraped off, was used as the material to prepare the immunogen solution. The antigen solution immediately after dialysis contained 0.19% the alcohol-

insoluble. It resembled the corresponding solution in *4.* chemical-qualitatively, but was almost Dische-negative. One of two antisera prepared (I) had antigen titre 5000 and antibody titre 32 and the other (II) the corresponding titres of 2500 and of 16.

II. Serological reactions

1. Precipitin test

The ring test was processed in two ways by the α- and β-Marrack procedures at 37°C. A 10 mg. portion of the in vitro antigens (reactionogens) (S. below) were dissolved in 1 cc. of physiological saline and further diluted with physiological saline progressively twofold. The antisera were diluted in most cases in a similar manner but with physiological saline containing 1.5% Arabic gum.

2. Complement-fixing test

This test was adapted for examining the antigenicity of the alcohol-soluble lipid components of the pig stomach mucosa.

The in vitro antigen (reactionogen). The pig stomach mucosa (ground) was maintained under 10 volumes of abs. denatured alcohol containing 3% CH_3OH in an incubator at 37°C for 1 week, with occasional agitation. The solution was separated and distilled in vacuo to a syrup and dried in a desiccator over $CaCl_2$. From 50 g. of the fresh material 5.6 g. of the extract were given. Next, 5 g. of it were dissolved in 100 cc. of abs. alcohol at 37°C which took 24 hours, followed by standing for several hours at room temperature (18°C) wherby a precipitate occurred. The precipitate was filtered off and the filtrate (96 cc.) was distilled in vacuo to a thick paste, which was subjected to a similar purification procedure twice more. 2.4 g. of a dry dark-brown putty-like substance were given—Cold-alcohol soluble fraction.

A 0.5% solution of the product in alcohol was applied as the original antigen solution, one of the anti-pig gastric mucosa-sera as the antiserum, a guinea-pig serum, diluted as to contain 2 complement units in 0.1 cc., as the complement solution, and a 2.5% beef erythrocyte suspension in an immune rabbit serum, possessing hemolysin titre 2400 and diluted, (0.25 cc. of the hemolytic system contained 7 units) as the hemolytic system. And the test was processed as follows: 0.25 cc. of the antigen solution diluted 40 or 30 times with physiological saline, 0.25 cc. of the antiserum, absorbed and progressively diluted, and 0.1 cc. of the complement solution were let combine together by standing at 37°C for 1 hour (shaken every 15 minutes), and after addition of the sensitized beef erythrocytes (0.25 cc. of the suspension), further stood at the same temperature for the same duration of time (shaken every 15 minutes) to examine the occurrence of hemolysis.

3. Absorption of the antisera with in vitro antigens

The absorption was made by adding a quantity of an in vitro antigen (See below) to a serum and standing the mixture at 37°C for 3 hours and overnight in an ice chamber. The precipitate was centrifuged off and the supernatant was examined whether or not complete absorption had been effected by addition of an additional portion of the antigen.

III. The in vitro antigens

1. Whole-pig gastric mucosa-antigen

The clear solution, obtained after extraction of the mucosa and dialysis in the manner in I, *i*., a), was condensed immediately, precipitated with alcohol, followed by washing with ether, and dried. The substance given was used as a whole as the in vitro antigen—Whole-pig stomach mucosa-antigen.

Besides, the high-molecular components which have been described in the first paper[1] and the alcohol-soluble above were employed as reactionogens from the same tissue.

2. Whole-antigen of the parts of the pig digestive tract other than stomach

The mucosa of the various parts of the digestive tract (oesophagus, duodenum, small intestine and colon) were separately treated as that of stomach to gain preparations comprising the physiological saline-extractible, alcohol-precipitable components—Whole-antigens.

3. Whole-pig kidney-antigen

The antigen corresponding to the Whole-pig stomach mucosa-antigen was prepared from the kidney cortex in a similar manner.

4. Whole-pig liver-antigen

The preparation corresponding to the stomach antigen in *1.* and the one obtained without dialysis were used.

5. Pig serum as an absorbent

Fresh normal pig serum was inactivated and after addition of 1/19 volume of physiological saline containing 5% phenol, preserved in an ampoule.

6. Whole-canine gastric mucosa-antigen

A mixture of the physiological saline-soluble, alcohol-insoluble components corresponding to Whole-pig gastric mucosa-antigen was separated from the canine stomach mucosa.

Results

1. Serological reactions of a rabbit anti-pig gastric mucosa-serum with the extract of the pig tissues and the components of the pig gastric mucosa

a) The details of preciption reaction of the antiserum unabsorbed are illustrated in Tables I, II and III. The immune rabbit serum gave the reaction not only with any of the various tissue extracts but also with pig blood serum, whereas no natural antibodies of those extracts were

TABLE I

Precipitin Reaction of Whole-Pig Gastric Mucosa-Antigen with the Anti-Pig Gastric Mucosa-Rabbit Serum, showing antigen titre of 1000×10^5 and antibody titre of 2^5

−: negative reaction, ±: faint r., +: definitely positive r.

Ditution of antiserum (n in $\times 2^n$)	Dilution of antigen (n in $\times 1000 \times 2^n$)								
	0	1	2	3	4	5	6	7	∞
0	+	+	+	+	+	+	±	−	−
1	+	+	+	+	+	+	±	−	−
2	+	+	+	+	+	+	±	−	−
3	+	+	+	+	+	+	±	−	−
4	+	+	+	+	+	±	−	−	−
5	+	+	+	+	−	−	−	−	−
6	−	−	−	−	−	−	−	−	−

TABLE II

Precipitin Reaction of the Various Whole-Antigens of Pig Tissues with Anti-Pig Gastric Mucosa-Rabbit Serum

The anti-serum was used without dilution. −: negative reaction, ±: faint r., +: difinitely positive r.

Whole-tissue-antigen (name of pig tissue)	Dilution of antigens (n in $\times 1000 \times 2^n$)								
	0	1	2	3	4	5	6	7	∞
Stomach mucosa	+	+	+	+	+	+	±	−	−
Oesophagus mucosa	+	+	+	+	+	+	±	−	−
Duodenum mucosa	+	+	+	+	+	+	±	−	−
Small-intestine mucosa	+	+	+	+	+	+	−	−	−
Colon mucosa	+	+	+	+	+	+	−	−	−
Liver	+	+	+	+	+	±	−	−	−
Kidney cortex	+	+	+	+	+	+	−	−	−

detected in normal rabbit serum.

b) Precipitin reaction of the antiserum, absorbed with pig blood serum or with pig serum and one of the extract of the tissues other than the stomach mucosa, with the various tissue extracts. Since the rabbit antiserum had been found to be completely absorbed with 0.23 volume of the pig serum, 1/4 the volume of the latter was used for absorption in this and following cases. Table IV shows the results given by the antiserum absorbed with the pig serum alone. All the Whole-antigens showed positive reaction, the gastric mucosa naturally proving most powerful. The extract of the lower parts of the digestive tract

TABLE III

Precipitin Reaction of Pig Serum, as an in vitro Antigen, with Anti-Pig Gastric Mucosa-Rabbit Serum

−: negative reaction, ±: faint r., +: definitely positive r.

Dilution of the rabbit antiserum (n in $\times 2^n$)	Dilution of antigen (n in $\times 10 \times 2^n$)													
	0	1	2	3	4	5	6	7	8	9	10	11	12	∞
0	+	+	+	+	+	+	+	+	+	+	+	+	−	−
1	+	+	+	+	+	+	+	+	+	+	+	+	−	−
2	+	+	+	+	+	+	+	+	+	+	+	+	−	−
3	+	+	+	+	+	+	+	−	−	−	−	−	−	−
4	+	+	+	+	+	+	+	−	−	−	−	−	−	−
5	−	−	−	−	−	−	−	−	−	−	−	−	−	−

TABLE IV

Precipitin Reaction of the Various Whole-Antigens of Pig Tissues with the Anti-Pig Gastric Mucosa-Rabbit Serum, absorbed with Normal Pig Serum

The antiserum was used without dilution. −: negative reaction, ±: faint r., +: definitely positive r.

Whole-tissue-antigen (name of pig tissue)	Dilution of antigens											
	n in $\times 100 \times 2^n$				n in $\times 1000 \times 2^n$							
	0	1	2	3	0	1	2	3	4	5	6	∞
Stomach mucosa	+	+	+	+	+	+	+	+	+ (16)*	±	−	−
Oesophagus mucosa	+	+	+	+	+	+	+	+	−	−	−	−
Duodenum mucosa	+	+	+	+	+	+	+	−	−	−	−	−
Small-intestine mucosa	+	+	+	+	+	±	−	−	−	−	−	−
Colon mucosa	+	+	+	+	+	−	−	−	−	−	−	−
Liver	+	±	−	−	−	−	−	−	−	−	−	−
Kidney cortex	+	+	±	−	−	−	−	−	−	−	−	−

* Antibody titre.

gave the reaction, with strengths decreasing with the increase of distance of the parts from stomach. The extracts from the liver and kidney also reacted though weak. The results embodied in Table V, which account for the precipitin reactions of the antiserum after double absorption with pig serum and one of the Whole-antigens, indicate that, when absorbed with the extract of the oesophagus besides the pig serum, the antiserum was able to give the reaction only with the Whole-stomach mucosa-antigen, but, when the antiserum had been absorbed with the extract

TABLE V

Precipitin Reaction of the Whole-Antigen of Various Pig Tissues with the Anti-Pig Gastric Mucosa-Rabbit Serum, double absorbed with Normal Pig Serum and One of the Whole-Antigens

The antiserum was used without dilution. −: negative reaction, ±: faint r., +: definitely positive r.

Whole-tissue-antigen (name of pig tissue)	Dilution of antigens										
	n in ×100×2ⁿ				n in ×1000×2ⁿ						
	0	1	2	3	0	1	2	3	4	5	∞
Antiserum absorbed with Whole-oesophagus mucosa-antigen besides pig serum											
Stomach mucosa	+	+	+	+	+	+	+ (16)*	−	−	−	−
Duodenum mucosa	−	−	−	−	−	−	−	−	−	−	−
Small-intestine mucosa	−	−	−	−	−	−	−	−	−	−	−
Colon mucosa	−	−	−	−	−	−	−	−	−	−	−
Liver	−	−	−	−	−	−	−	−	−	−	−
Kidney cortex	−	−	−	−	−	−	−	−	−	−	−
Antiserum absorbed with Whole-duodenum mucosa-antigen besides pig serum											
Stomach mucosa	+	+	+	+	+	+	+	+	−	−	−
Oesophagus mucosa	+	−	−	−	−	−	−	−	−	−	−
Small-intestine mucosa	+	+	+	+	+	−	−	−	−	−	−
Colon mucosa	+	+	+	+	+	−	−	−	−	−	−
Liver	±	−	−	−	−	−	−	−	−	−	−
Kidney cortex	+	±	−	−	−	−	−	−	−	−	−
Antiserum absorbed with Whole-small-intestine mucosa-antigen besides pig serum											
Stomach mucosa	+	+	+	+	+	+	+	+	−	−	−
Oesophagus mucosa	+	+	+	+	+	+	+	±	−	−	−
Duodenum mucosa	+	+	+	−	−	−	−	−	−	−	−
Colon mucosa	+	+	+	+	+	−	−	−	−	−	−
Liver	+	−	−	−	−	−	−	−	−	−	−
Kidney cortex	±	−	−	−	−	−	−	−	−	−	−
Antiserum absorbed with Whole-colon mucosa-antigen besides pig serum											
Stomach mucosa	+	+	+	+	+	+	+	+	+	−	−
Oesophagus mucosa	+	+	+	+	+	+	+	±	−	−	−
Duodenum mucosa	+	+	+	+	+	+	±	−	−	−	−
Small-intestine mucosa	+	+	+	+	+	−	−	−	−	−	−
Liver	±	−	−	−	−	−	−	−	−	−	−
Kidney cortex	+	+	−	−	−	−	−	−	−	−	−
Antiserum absorbed with Whole-liver-antigen besides pig serum											
Stomach mucosa	+	+	+	+	+	+	+	+	+	−	−
Oesophagus mucosa	+	+	+	+	+	+	+	+	−	−	−
Duodenum mucosa	+	+	+	+	+	+	+	−	−	−	−
Small-intestine mucosa	+	+	+	+	+	−	−	−	−	−	−
Colon mucosa	+	+	+	+	+	−	−	−	−	−	−
Kidney cortex	±	−	−	−	−	−	−	−	−	−	−

TABLE V (Continued)

Whole-tissue-antigen (name of pig tissue)	Dilution of antigens										
	n in $\times 100 \times 2^n$				n in $\times 1000 \times 2^n$						
	0	1	2	3	0	1	2	3	4	5	∞
Antiserum absorbed with Whole-kidney cortex-antigen besides pig serum.											
Stomach mucosa	+	+	+	+	+	+	+	+	+	−	−
Oesophagus mucosa	+	+	+	+	+	+	+	+	−	−	−
Duodenum mucosa	+	+	+	+	+	+	+	−	−	−	−
Small-intestine mucosa	+	+	+	+	+	−	−	−	−	−	−
Colon mucosa	+	+	+	+	+	−	−	−	−	−	−
Liver	−	−	−	−	−	−	−	−	−	−	−

* Antibody titre.

of the mucosa of duodenum or small intestine or colon, there remained in it a pretty large amount of antibodies not only reactive with the Whole-antigen of stomach mucosa but also reactive with the antigens of the other tissues. The absorption with the extracts of liver and of kidney (cortex) effected no distinct influence.

c) The antiserum, absorbed with pig serum or successively with pig serum and one of the tissue extracts (Whole tissue-antigens) excepting the one of the stomach mucosa, was examined regarding the precipitin reaction with high molecular, water-soluble components of the gastric mucosa (Proteins I and II, the polyuronic acid mixture, the pentosenucleic acid and the group carbohydrate described in First Paper). The normal rabbit serum showed no precipitin reaction with any of the components. The results in Table VI illustrate: 1) the antiserum, absorbed with pig serum, reacted with all the stomach mucosa components remarkably; 2) Whole-oesophagus mucosa-antigen absorbed the antibody of all the stomach mucosa components other than the pentosenucleic acid; 3) the Whole-antigens of the other tissues could not entirely absorb the antibodies which Whole-oesophagus mucosa-antigen did, the degree of absorption lowering generally in the order below, the extract of the last tissue being the least effective, just resembling the relationship in the reaction between this antiserum and the Whole-tissue-antigens (See b)).

duodenum mucosa; small-intestine mucosa; colon mucosa; liver; kidney cortex

By none of those tissue extracts the antibody of the stomach pentosenucleic acid was absorbed.

d) The antigenicity of the cold-alcohol soluble fraction of the stomach mucosa was examined by the complement fixation (Regarding the technique, s. above). Table VIIA shows the minimum anti-comple-

TABLE VI

Precipitin Reaction of Whole-Antigen and Components of Pig Stomach Mucosa with the Anti-Pig Gastric Mucosa-Rabbit Serum, absorbed with Normal Pig Serum or with Normal Pig Serum and one of the Whole-Antigens of the Pig Tissues other than Stomach Mucosa

The antiserum was used without dilution. −: negative reaction, ±: faint r., +: definitely positive r. Figures in parentheses express the antibody titre.

Antigen	Dilution of antigens														
	n in $\times 100 \times 2^n$				n in $\times 1000 \times 2^n$										
	0	1	2	3	0	1	2	3	4	5	6	7	8	9	10	∞
Antiserum absorbed with pig serum																
Whole-stomach mucosa-antigen	+	+	+	+	+	+	+	+	+ (16)	−	−	−	−	−	−	−
Protein I*	+	+	+	+	+	+	+ (1)	−	−	−	−	−	−	−	−	−
Protein II*	+	+	+	+	+	+	+ (8)	−	−	−	−	−	−	−	−	−
PNA*	+	+	+	+	+	+	+	+ (16)	−	−	−	−	−	−	−	−
Polyuronic Acids*	+	+	+	+	+	+	+	+ (8)	−	−	−	−	−	−	−	−
Group Carbohydrate*	+	+	+	+	+	+	+	+	+	+	+	+	+	+ (8)	−	−
Antiserum absorbed with pig serum and Whole-oesophagus mucosa-antigen																
Whole-stomach mucosa-antigen	+	+	+	+	+	+	+ (8)	−	−	−						−
Protein I	−	−	−	−	−	−	−	−	−	−						−
Protein II	−	−	−	−	−	−	−	−	−	−						−
PNA	+	+	+	+	+	+	+	+ (8)	−	−						−
Polyuronic Acids	−	−	−	−	−	−	−	−	−	−						−
Group Carbohydrate	−	−	−	−	−	−	−	−	−	−						−
Antiserum absorbed with pig serum and Whole-duodenum mucosa-antigen																
Whole-stomach mucosa-antigen	+	+	+	+	+	+	+	−	−							−
Protein I	±	−	−	−	−	−	−	−	−							−
Protein II	+	+	−	−	−	−	−	−	−							−
PNA	+	+	+	+	+	+	+	±	−							−
Polyuronic Acids	+	+	+	+	+	−	−	−	−							−
Group Carbohydrate	+	+	−	−	−	−	−	−	−							−
Antiserum absorbed with pig serum and Whole-small-intestine mucosa-antigen																
Whole-stomach mucosa-antigen	+	+	+	+	+	+	+	±	−							−
Protein I	+	+	±	−	−	−	−	−	−							−
Protein II	+	+	±	−	−	−	−	−	−							−
PNA	+	+	+	+	+	+	+	+	−							−
Polyuronic Acids	+	+	±	±	−	−	−	−	−							−
Group Carbohydrate	+	+	+	±	±	−	−	−	−							−

TABLE VI (Continued)

Antigen	Dilution of antigens															
	n in $\times 100 \times 2^n$				n in $\times 1000 \times 2^n$											
	0	1	2	3	0	1	2	3	4	5	6	7	8	9	10	∞
Antiserum absorbed with pig serum and Whole-colon mucosa-antigen																
Whole-stomach mucosa-antigen	+	+	+	+	+	+	+	±	−							−
Protein I	+	+	+	−	−	−	−	−	−							−
Protein II	+	+	+	+	−	−	−	−	−							−
PNA	+	+	+	+	+	+	+	+	−							−
Polyuronic Acids	+	+	+	+	+	+	+	−	−							−
Group Carbohydrate	+	+	+	+	+	+	±	−	−							−
Antiserum absorbed with pig serum and Whole-liver-antigen																
Whole-stomach mucosa-antigen	+	+	+	+	+	+	+	+	±	−	−	−				−
Protein I	+	+	+	±	−	−	−	−	−	−	−	−				−
Protein II	+	+	+	+	+	+	+	−	−	−	−	−				−
PNA	+	+	+	+	+	+	+	+	−	−	−	−				−
Polyuronic Acids	+	+	+	+	+	+	−	−	−	−	−	−				−
Group Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	−				−
Antiserum absorbed with pig serum and Whole-kidney cortex-antigen																
Whole-stomach mucosa-antigen	+	+	+	+	+	+	+	+	−	−	−	−	−	−		−
Protein I	+	+	+	+	−	−	−	−	−	−	−	−	−	−		−
Protein II	+	+	+	+	+	+	+	−	−	−	−	−	−	−		−
PNA	+	+	+	+	+	+	+	+	−	−	−	−	−	−		−
Polyuronic Acids	+	+	+	±	−	−	−	−	−	−	−	−	−	−		−
Group Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	+	+	−		−

* Cf. the First Paper.

mentary dose of the antigen itself. Since the antigen solution diluted more than 10 times gave rise to no hemolysis, it was diluted 30 and 40 times for the main test. Table VIIB demonstrates that, when absorbed with pig serum alone, the antiserum inhibited hemolysis, but not after further absorption with the Whole-oesophagus mucosa-antigen, so that the alcohol-soluble fraction of the stomach mucosa is considered to be able to form an antibody in rabbits, which, however, is not strictly specific to that tissue.

2. An anti-pig liver-serum did not give precipitin reaction with the high-molecular components of pig stomach mucosa

Anti-pig liver-serum II above was employed for the present assay. The precipitin reaction of the antiserum with Whole-pig liver-antigen is illustrated in Table VIIIA. The antigen and antibody titre of the anti-serum before absorption with pig serum were 1000×2^5 and 2^5 respectively

TABLE VII

A. Occurrence of Hemolysis when Cold-alcohol Soluble Fraction of the Pig Gastric Mucosa as an in vitro Antigen, Complement and Sensitized Erythrocytes were mixed together without Antibody

The test was processed as the complement-fixing test (S. text), replacing the diluted antiserum with physiological saline. −: no hemolysis, ++: marked hemol., ###: complete hemol.

Dilution of the original antigen sol. (1:)	5	10	15	20	25	30	35	40	45	50	∞
Occurrence of hemolysis	−	−	++	###	###	###	###	###	###	###	###

B. Complement-fixing Test of the Antigen in A. together with the Anti-Pig Gastric Mucosa-Serum

−: no hemolysis, +: slight hemol., ++: marked hemol., ###: complete hemol.

i) The antiserum absorbed with pig serum

The antigen and antibody titre of the absorbed antiserum in precipitin reaction with Whole-pig gastric mucosa-antigen were 1000×2^4 and 2^4 respectively (See Table IV)

Dilution of antiserum (1:)	10	2× 10	4× 10	8× 10	1.6 ×10²	3.2 ×10²	6.4 ×10²	1.28 ×10³	2.56 ×10³	∞	without antigen (dilution of antiserum: 1:10)
The original antigen solution (in alcohol, 0.5%) was diluted 40 times											
Occurrence of hemolysis	−	−	−	−	+	###	###	###	###	###	###
The original antigen solution was diluted 30 times											
Occurrence of hemolysis	−	−	−	−	+	###	###	###	###	###	###

ii) The antiserum double absorbed with pig serum and Whole-pig oesophagus mucosa-antigen

The antigen and antibody titre of the absorbed antiserum in precipitin reaction with Whole-pig gastric mucosa-antigen were 1000×2^2 and 2^4 respectively (See Table V).

Dilution of antiserum (1:)	10	2× 10	4× 10	8× 10	1.6 ×10²	3.2 ×10²	6.4 ×10²	1.28 ×10³	∞	without antigen (dilution of antiserum: 1:10)
The original antigen solution (in alcohol, 0.5%) was diluted 40 times										
Occurrence of hemolysis	++	###	###	###	###	###	###	###	###	###
The original antigen solution was diluted 30 times										
Occurrence of hemolysis	++	###	###	###	###	###	###	###	###	###

TABLE VI (Continued)

Antigen	Dilution of antigens														
	n in $\times 100 \times 2^n$				n in $\times 1000 \times 2^n$										
	0	1	2	3	0	1	2	3	4	5	6	7	8	9	10	∞
Antiserum absorbed with pig serum and Whole-colon mucosa-antigen																
Whole-stomach mucosa-antigen	+	+	+	+	+	+	+	±	−							−
Protein I	+	+	+	−	−	−	−	−	−							−
Protein II	+	+	+	+	−	−	−	−	−							−
PNA	+	+	+	+	+	+	+	+	−							−
Polyuronic Acids	+	+	+	+	+	+	+	−	−							−
Group Carbohydrate	+	+	+	+	+	+	±	−	−							−
Antiserum absorbed with pig serum and Whole-liver-antigen																
Whole-stomach mucosa-antigen	+	+	+	+	+	+	+	+	±	−	−	−				−
Protein I	+	+	+	±	−	−	−	−	−	−	−	−				−
Protein II	+	+	+	+	+	+	+	−	−	−	−	−				−
PNA	+	+	+	+	+	+	+	+	−	−	−	−				−
Polyuronic Acids	+	+	+	+	+	+	−	−	−	−	−	−				−
Group Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	−				−
Antiserum absorbed with pig serum and Whole-kidney cortex-antigen																
Whole-stomach mucosa-antigen	+	+	+	+	+	+	+	+	−	−	−	−	−	−		−
Protein I	+	+	+	+	−	−	−	−	−	−	−	−	−	−		−
Protein II	+	+	+	+	+	+	+	−	−	−	−	−	−	−		−
PNA	+	+	+	+	+	+	+	+	−	−	−	−	−	−		−
Polyuronic Acids	+	+	+	±	−	−	−	−	−	−	−	−	−	−		−
Group Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	+	+	−		−

* Cf. the First Paper.

mentary dose of the antigen itself. Since the antigen solution diluted more than 10 times gave rise to no hemolysis, it was diluted 30 and 40 times for the main test. Table VIIB demonstrates that, when absorbed with pig serum alone, the antiserum inhibited hemolysis, but not after further absorption with the Whole-oesophagus mucosa-antigen, so that the alcohol-soluble fraction of the stomach mucosa is considered to be able to form an antibody in rabbits, which, however, is not strictly specific to that tissue.

2. An anti-pig liver-serum did not give precipitin reaction with the high-molecular components of pig stomach mucosa

Anti-pig liver-serum II above was employed for the present assay. The precipitin reaction of the antiserum with Whole-pig liver-antigen is illustrated in Table VIIIA. The antigen and antibody titre of the anti-serum before absorption with pig serum were 1000×2^5 and 2^5 respectively

TABLE VII

A. Occurrence of Hemolysis when Cold-alcohol Soluble Fraction of the Pig Gastric Mucosa as an in vitro Antigen, Complement and Sensitized Erythrocytes were mixed together without Antibody

The test was processed as the complement-fixing test (S. text), replacing the diluted antiserum with physiological saline. −: no hemolysis, +: marked hemol., ±: complete hemol.

Dilution of the original antigen sol. (1:)	5	10	15	20	25	30	35	40	45	50	∞
Occurrence of hemolysis	−	−	+	±	±	±	±	±	±	±	±

B. Complement-fixing Test of the Antigen in A. together with the Anti-Pig Gastric Mucosa-Serum

−: no hemolysis, +: slight hemol., +: marked hemol., ±: complete hemol.

i) The antiserum absorbed with pig serum

The antigen and antibody titre of the absorbed antiserum in precipitin reaction with Whole-pig gastric mucosa-antigen were 1000×2^4 and 2^4 respectively (See Table IV)

Dilution of antiserum (1:)	10	2× 10	4× 10	8× 10	1.6 ×10²	3.2 ×10²	6.4 ×10²	1.28 ×10³	2.56 ×10³	∞	without antigen (dilution of antiserum: 1:10)
The original antigen solution (in alcohol, 0.5%) was diluted 40 times											
Occurrence of hemolysis	−	−	−	−	+	±	±	±	±	±	±
The original dntigen solution was diluted 30 times											
Occurrence of hemolysis	−	−	−	−	+	±	±	±	±	±	±

ii) The antiserum double absorbed with pig serum and Whole-pig oesophagus mucosa-antigen

The antigen and antibody titre of the absorbed antiserum in precipitin reaction with Whole-pig gastric mucosa-antigen were 1000×2^2 and 2^4 respectively (See Table V).

Dilution of antiserum (1:)	10	2× 10	4× 10	8× 10	1.6 ×10²	3.2 ×10²	6.4 ×10²	1.28 ×10³	∞	without antigen (dilution of antiserum: 1:10)
The original antigen solution (in alcohol, 0.5%) was diluted 40 times										
Occurrence of hemolysis	+	±	±	±	±	±	±	±	±	±
The original antigen solution was diluted 30 times										
Occurrence of hemolysis	+	±	±	±	±	±	±	±	±	±

TABLE VIII

A. Precipitin Reaction of Anti-Pig Liver-Rabbit Serum with Whole-Pig Liver-Antigen

−: negative reaction, ±: faint r., +: definitely positive r.

Dilution of antiserum (n in $\times 2^n$)	Dilution of antigen (n in $\times 1000 \times 2^n$)							
	0	1	2	3	4	5	6	∞
The antiserum not absorbed								
0	+	+	+	+	+	+	−	−
1	+	+	+	+	+	+	−	−
2	+	+	+	+	+	+	−	−
3	+	+	+	+	+	−	−	−
4	+	+	+	+	+	−	−	−
5	+	+	+	+	−	−	−	−
6	±	±	−	−	−	−	−	−
The antiserum absorbed with pig serum								
0	+	+	+	+	+	−	−	−
1	+	+	+	+	+	−	−	−
2	+	+	+	+	+	−	−	−
3	+	+	+	+	−	−	−	−
4	+	+	+	+	−	−	−	−
5	+	+	+	−	−	−	−	−
6	−	−	−	−	−	−	−	−

B. The Reaction of the Same Antiserum, absorbed with Pig Serum, with Whole-Antigen and Components of Pig Stomach Mucosa

The antiserum was used without dilution. −: negative reaction.

Antigen	Dilution of antigens						
	n in $\times 100 \times 2^n$				n in $\times 1000 \times 2^n$		
	0	1	2	3	0	1	∞
Whole-stomach mucosa-antigen	−	−	−	−	−	−	−
Protein I	−	−	−	−	−	−	−
Protein II	−	−	−	−	−	−	−
PNA	−	−	−	−	−	−	−
Polyuronic Acids	−	−	−	−	−	−	−
Group Carbohydrate	−	−	−	−	−	−	−

and those after the absorption 1000×2^4 and 2^5 respectively. The antiserum treated with pig serum gave the positive reaction neither with Whole-pig stomach mucosa-antigen nor with the separated components

TABLE IX

A. Precipitin Reaction of Anti-Pig Kidney Cortex-Rabbit Serum with Whole-Pig Kidney Cortex-Antigen

−: negative reaction, ±: faint r. +: definitely positive r.

Dilution of antiserum (n in $\times 2^n$)	Dilution of antigen (n in $\times 1000 \times 2^n$)							
	0	1	2	3	4	5	6	∞
The antiserum not absorbed								
0	+	+	+	+	+	+	−	−
1	+	+	+	+	+	+	−	−
2	+	+	+	+	+	+	−	−
3	+	+	+	+	+	±	−	−
4	+	+	+	+	±	−	−	−
5	+	+	+	+	−	−	−	−
6	−	−	−	−	−	−	−	−
The antiserum absorbed with pig serum								
0	+	+	+	+	−	−	−	−
1	+	+	+	+	−	−	−	−
2	+	+	+	+	−	−	−	−
3	+	+	+	−	−	−	−	−
4	−	−	−	−	−	−	−	−
5	−	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−	−

B. The Reaction of the Same Antiserum, absorbed with Pig Serum, with Whole-Antigen and Components of Pig Stomach Mucosa

The antiserum was used without dilution. −: negative reaction.

Antigen	Dilution of antigens						
	n in $\times 100 \times 2^n$				n in $\times 1000 \times 2^n$		
	0	1	2	3	0	1	∞
Whole-stomach mucosa-antigen	−	−	−	−	−	−	−
Protein I	−	−	−	−	−	−	−
Protein II	−	−	−	−	−	−	−
PNA	−	−	−	−	−	−	−
Polyuronic Acids	−	−	−	−	−	−	−
Group Carbohydrate	−	−	−	−	−	−	−

of the tissue even at a dilution of the antigens of 1:100 (Table VIIIB).

3. Experiments similar to immediately above were carried out by replacing the anti-pig kidney (cortex)-serum for the anti-pig liver-serum

Table X

A. Precipitin Reaction of Anti-Canine Gastric Mucosa-Rabbit Serum (I) with Whole-Canine Gastric Mucosa-Antigen

−: negative reaction, ±: faint r., +: definitely positive r.

Dilution of antiserum (n in 2^n)	Dilution of antigen (n in $\times 1000 \times 2^n$)							
	0	1	2	3	4	5	6	∞
The antiserum not absorbed								
0	+	+	+	+	+	±	−	−
1	+	+	+	+	+	±	−	−
2	+	+	+	+	+	−	−	−
3	+	+	+	+	+	−	−	−
4	+	+	+	+	+	−	−	−
5	−	±	±	±	−	−	−	−
6	−	−	−	−	−	−	−	−
The antiserum absorbed with dog serum								
0	+	+	+	+	±	−	−	−
1	+	+	+	+	±	−	−	−
2	+	+	+	+	±	−	−	−
3	+	+	+	±	−	−	−	−
4	±	±	±	−	−	−	−	−
5	−	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−	−

B. The Reaction of the Same Antiserum, absorbed with Dog Serum, with Whole-Antigen and Components of Pig Stomach Mucosa

The antiserum was used without dilution. −: negative reaction, ±: faint r., +: definitely positive r.
Figures in parentheses express antibody titre.

Antigen	Dilution of antigens									
	n in $\times 100 \times 2^n$				n in $\times 1000 \times 2^n$					
	0	1	2	3	0	1	2	3	4	∞
Whole-pig stomach mucosa-antigen	+	+	+	+	± (4)	−	−	−	−	−
Protein I	± (1)	−	−	−	−	−	−	−	−	−
Protein II	± (1)	−	−	−	−	−	−	−	−	−
PNA	+	+	+	+	+	+	+ (4)	−	−	−
Polyuronic Acids	±	± (1)	−	−	−	−	−	−	−	−
Group Carbohydrate	+	+	+	+	+	+	+	± (1)	−	−

The results are embodied in Tables IXA and IXB. The antigen and antibody titre of the antiserum in the reaction with the Whole-kidney cortex-antigen were 1000×2^5 and 2^5 respectively before absorption with pig serum and 1000×2^3 and 2^3 respectively after the absorption. The antiserum absorbed with pig serum gave no positive precipitin reaction with the antigens of the pig stomach mucosa as above.

4. The precipitin reaction of the anti-canine gastric mucosa-sera

Whole-canine gastric mucosa-antigen reacted distinctly with the antisera, as such as well as after absorption with dog serum. Of the pig stomach mucosa components, Proteins I and II and Polyuronic Acids reacted only little with the antisera treated with dog serum, but, contrariwise, PNA and Group Carbohydrate eminently. Table X shows the results given by the antiserum I.

Summary and Conclusion

1, Rabbit antisera of the physiological saline extracts of stomach-, oesophagus-, duodenum-, small intestine- and colon-mucosa, liver and kidney-cortex of pig and of the canine gastric mucosa were prepared, and the serological tests of those sera with the extracts of those tissues and the components (proteins, pentosenucleic acid, polyuronic acids, group mucopolysaccharide, cold-alcohol soluble fraction) of pig gastric mucosa were carried out.

2. The anti-pig gastric mucosa serum, absorbed with pig serum, gave the serological (precipitin- and complement-fixing-) reactions not only with the saline extract and cold-alcohol soluble fraction of this mucosa but also with the saline extract of other pig tissues, the extract of the lower parts of the digestive tract reacting with strengths decreasing with the increase in distance of the parts from the stomach. After further absorption with the extract of pig oesophagus mucosa, it reacted with the gastric mucosa extract but not with those obtained from the other tissues. When the extract of the oesophagus mucosa was substituted with that of another part of the pig digestive tract for absorption, the absorbed serum gave precipitin reaction with the extracts of the various parts of the digestive tract (exclusive of the one used for absorption). And, when absorption was effected with the extract of pig liver besides pig serum, the antiserum lost the potency of giving the reaction with the extract of pig kidney and *vice versa*, but absorption with these extracts was of no influence upon the potency of the antiserum of giving the reaction with the extract of the other pig tissues,

3. The anti-pig gastric mucosa-serum absorbed with pig serum and oesophagus extract precipitated only the pentosenucleic acid among the water-soluble, high-molecular pig gastric mucosa components and,

at that, at the same concentration of the nucleic acid as when the antiserum had been absorbed with pig serum alone. When absorbed with another pig tissue extract besides pig serum, the antiserum reacted also with the pig gastric mucosa components other than the pentosenucleic acid, intensity varying according to the absorbent as had been suggested by the precipitin reaction of the antiserum, absorbed with pig serum alone, with the extract of the other tissues, and the antigen dilution, where the pentosenucleic acid reacts, was not altered by any of the tissue extracts employed for absorption. The anti-pig gastric mucosa-serum after absorption with both pig serum and pig oesophagus mucosa extract did not give the complement-fixing reaction with the cold-alcohol soluble fraction of the pig gastric mucosa, unlike after absorption with pig serum alone. The findings are suspected to be an evidence that the pentosenucleic acid of the pig gastric mucosa is the sole antigen specific to this tissue.

4. The water-soluble pig gastric mucosa components did not give precipitin reaction with the anti-pig liver- and anti-pig kidney-serum, but did with the anti-canine gastric mucosa-serum absorbed with canine serum, the pentosenucleic acid and group carbohydrate reacting strongly and the proteins and polyuronic acids faintly.

5. Taken altogether, the pentosenucleic acid of gastric mucosa in general may be plausibly assumed to be extremely organ-specific in distinction from the other components of this tissue.

Through the Grant Committee for Scientific Researches was given a grant from the Education Department in aid to us, which is gratefully acknowledged. H. Masamune.

References

1) Tokutomi, Tohoku J. Exp. Med., this volume, p. 163.

ANTIQUE